Fluidigm® Real-Time PCR Analysis Software v2.1

User Guide

Limited License and Disclaimer for Fluidigm® Systems with Dynamic Array™ IFCs and Digital Array™ IFCs

Except as expressly set forth herein, no right to copy, modify, distribute, make derivative works of, publicly display, make, have made, offer to sell, sell, use, or import a Fluidigm system or any other product is conveyed or implied with the purchase of a Fluidigm system (including the BioMark™ system, EP1 system, or any components thereof), Dynamic Array IFCs and Digital Array IFCs (integrated fluidic circuits/microfluidic chips with or without a carrier), IFC controller, software, reagents, or any other items provided hereunder. This limited license permits only the use by the buyer of the particular product(s), in accordance with the written instructions provided therewith in the User Guide, that the buyer purchases from Fluidigm or its authorized representative(s). Except to the extent expressly approved in writing by Fluidigm or its authorized representative(s), the purchase of any Fluidigm product(s) does not by itself convey or imply the right to use such product(s) in combination with any other product(s). In particular, (i) no right to make, have made, or distribute other instruments, Dynamic Array IFCs and Digital Array IFCs, software, or reagents is conveyed or implied by the purchase of the Fluidigm system, (ii) no right to make, have made, import, distribute, or use the Fluidigm system is conveyed or implied by the purchase of instruments, software, reagents, or Dynamic Array IFCs and Digital Array IFCs from Fluidigm or otherwise, and (iii) except as expressly provided in the User Guide, the buyer may not use and no right is conveyed to use the Fluidigm system in combination with instruments, software, reagents, or dynamic arrays and digital arrays unless all component parts have been purchased from Fluidigm or its authorized representative(s). For example, purchase of a Fluidigm system and/or the IFC controller conveys no right or license to patents covering the Dynamic Array IFCs and Digital Array IFCs or their manufacture, such as 5,376,252, 6,408,878, 6,645,432, 6,719,868, 6,793,753, 6,929,030; U.S. Patent Application Nos. 2005/0084421, 2005/0112882, 2005/0129581, 2005/0145496, 2005/0214173; and EP Patent Nos. 0527905 and 1065378. Fluidigm Dynamic Array IFCs and Digital Array IFCs delivered hereunder are licensed for one (1) time use and may not be reused, refurbished, or resold. All Fluidigm products are licensed to the buyer for research use only. The products do not have FDA or other similar regulatory body approval. The buyer may not use the Fluidigm system, any component parts thereof, or any other Fluidigm products in any setting requiring FDA or similar regulatory approval or exploit the products in any manner not expressly authorized in writing by Fluidigm in advance. No other licenses are granted, expressed or implied. Please refer to the Fluidigm website at www.fluidigm.com for updated license terms.

Fluidigm, the Fluidigm logo, BioMark, Dynamic Array, and Digital Array are trademarks or registered trademarks of Fluidigm Corporation in the U.S. and/or other countries. TaqMan® is a registered trademark of Roche Molecular Systems in certain countries; all other trademarks are the sole property of their respective owners.

Thermal Cycler

The BioMark System contains a licensed thermal cycler supplied by Eppendorf AG.

Recommended Reagents

Fluidigm recommends that you use only TaqMan® dual-labeled probes and/or other licensed PCR assay reagents from authorized sources. If you have any questions regarding whether you have a license to use particular reagents in PCR systems, you should contact the appropriate licensor and obtain clarification and their permission if necessary. For example, certain probes and their use may be covered by one or more patents held by Applied Biosystems and/or Roche Molecular Systems, which may be contacted at the Director of Licensing at Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404 or the Licensing Department, Roche Molecular Systems, Inc., 1145 Atlantic Avenue, Alameda, California 94501.

Fluidigm® Product Patent Notice

Fluidigm® products including IFCs (integrated fluidic circuits/microfluidic chips with or without a carrier) such as Dynamic Array IFCs and Digital Array IFCs, the IFC controller, and the Fluidigm system (BioMark™ system, EP1 system, readers, thermal cycler, etc.) and methods for reading and controlling the Dynamic Array IFCs and Digital Array IFCs and/or their use and manufacture may be covered by one or more of the following patents and patent applications owned by Fluidigm Corporation and/or sold under license from California Institute of Technology, Gyros AB, and other entities: U.S. Patent Nos. 5,376,252, 6,408,878, 6,645,432, 6,719,868, 6,793,753, 6,929,030, 7,195,670, 7,216,671, 7,307,802, 7,323,143, 7,476,363; U.S. Patent Application Nos. 2005/0084421, 2005/0112882, 2005/0129581, 2005/0145496, 2005/0201901, 2005/0214173, 2005/0252773; EP Patent Nos. 0527905 and 1065378; and additional issued and pending patents in the U.S. and other countries.

Please refer to the User Guide for certain license terms for Fluidigm system products and the Fluidigm website at www.fluidigm.com for updates.

[Version 3.8]

No right to modify, copy, use, or distribute Fluidigm software is provided except in conjunction with the instrument delivered hereunder and only by the end user receiving such instrument. In cases where the software and the associated instrument are beta test systems, NO WARRANTIES ARE PROVIDED, EXPRESSED OR IMPLIED. ALL WARRANTIES, INCLUDING THE IMPLIED WARRANTIES OF FITNESS FOR PURPOSE, MERCHANTABILITY, AND NON-INFRINGMENT ARE EXPRESSLY DISCLAIMED.

By continuing the installation process, user agrees to these terms. Please refer to the full text of the software license agreement supplied with the installation media for this application.

Every effort has been made to avoid errors in the text, diagrams, illustrations, figures, and screen captures. However, Fluidigm assumes no responsibility for any errors that may appear in this publication.

It is Fluidigm's policy to improve products as new techniques and components become available. Therefore, Fluidigm reserves the right to change specifications at any time.

Information in this manual is subject to change without notice. Fluidigm assumes no responsibility for any errors or omissions. In no event shall Fluidigm be liable for any damages in connection with or arising from the use of this manual.

Contacting Fluidigm

For Technical Support, send email to: **TechSupport@fluidigm.com**

Phone in United States: **1.866.FLUIDLINE** (1.866.358.4354)

Outside the United States: 650.266.6100

On the Internet: **www.fluidigm.com/support**

Fluidigm Corporation

7000 Shoreline Court, Suite 100

South San Francisco, CA 94080

PN 68000088, Rev. C

Contents

Chapter 1 BioMark System

Chapter 2 Using Real-Time PCR Analysis Software

Appendix A 48.48 Dynamic Array™ IFC Real-Time PCR Workflow

Appendix B 96.96 Dynamic Array™ IFCs Real-Time PCR Workflow

Appendix C 48.48 PCR Installation Test

BioMark System 1

In this chapter:

Gene Expression Analysis Overview

Genes control cellular activity through a process known as gene expression. Gene expression begins when a cell transcribes a section of a gene's DNA to create a nucleic acid sequence, known as messenger RNA. This messenger RNA may then be translated by the cell into a protein. Messenger RNA can be detected and quantified by performing real-time quantitative polymerase chain reaction (qPCR) tests (assays). Gene expression analysis involves determining which genes are active by measuring messenger RNA levels in a blood or tissue sample. These results can be correlated with disease activity and clinical outcomes. As multiple genes are potentially involved in most biological processes, gene expression analysis typically requires assaying the expression levels of many genes simultaneously across many samples.

Real-Time qPCR

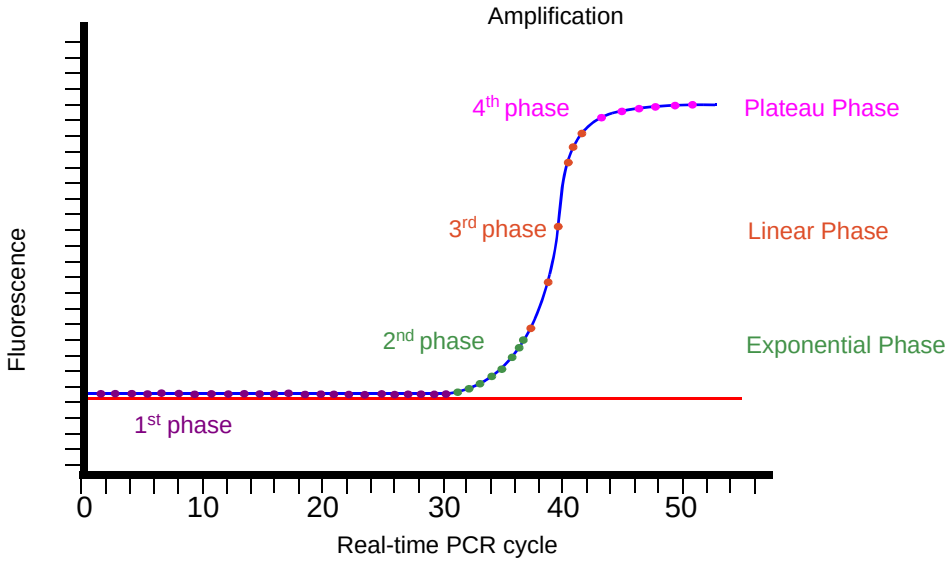
Real-time quantitative PCR (qPCR) is a powerful technique for quantifying changes in gene expression by producing millions of copies of specific, targeted regions of complementary DNA (cDNA) that has been reverse transcribed from messenger RNA (mRNA).

Advantages of Real Time qPCR

Historically, qPCR has been a time-consuming process because of the time it took to get gel-based end-point-measured (plateau phase) results. These results tended to be less accurate, and did not have as wide a dynamic range as real-time PCR. With the advent of quantitative data collection during the exponential phase of PCR, real-time quantification is a reality.

PCR Fundamentals

To appreciate the advantages of real-time PCR, a short review of PCR fundamentals is in order. At the start of a PCR reaction, reagents are in excess, both template and product are at low enough concentrations that product renaturation does not compete with primer binding, and amplification proceeds at a constant, exponential rate. The point at which the reaction rate ceases to be exponential and enters a linear phase of amplification is variable, and, at the plateau phase, the amplification rate drops to near zero.



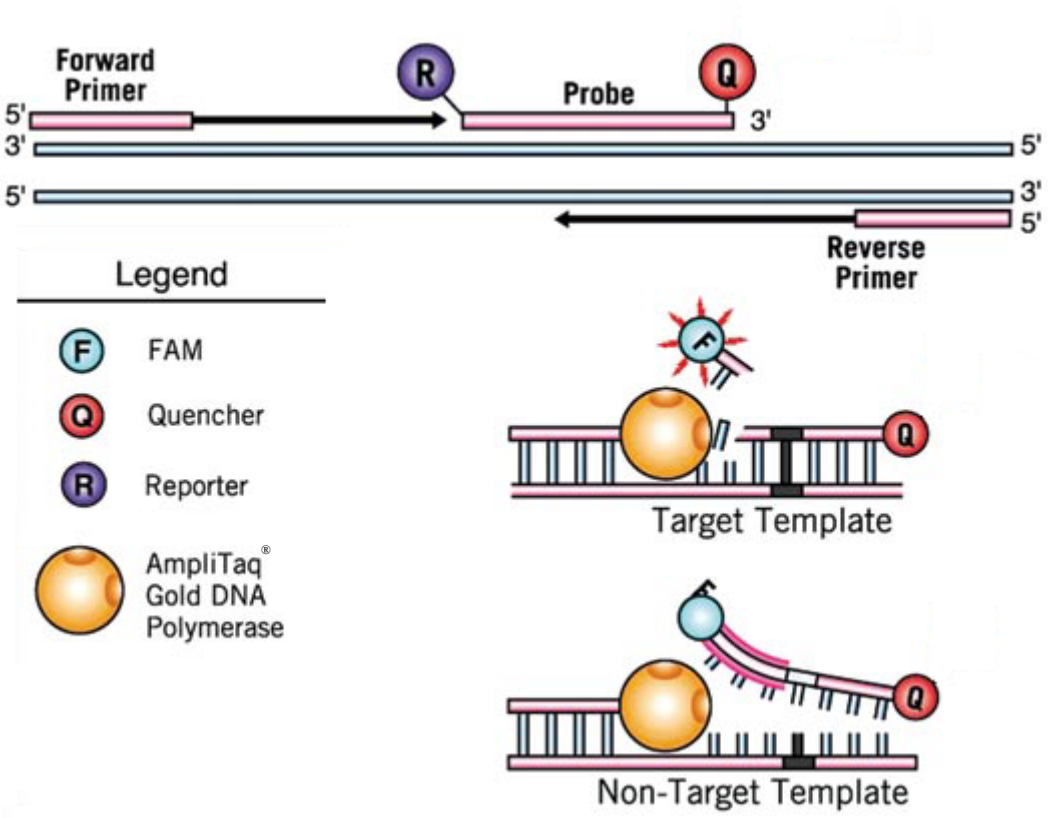
The Exponential Phase

To ensure accuracy and precision, quantitative data is best when collected at a point in which every reaction is in the exponential phase of amplification—this being the only phase in which amplification is easily reproducible.

Advantages of Real-Time qPCR TaqMan® Chemistry

The BioMark™ System uses dual-labeled probes, such as TaqMan® probes, for real-time qPCR amplification.

Dual-labeled probes are oligonucleotides that contain a fluorescent reporter dye on the 5' base, and a quencher located on the 3' base. When irradiated, the excited fluorescent reporter dye transfers energy to the nearby quencher molecule rather than fluorescing, resulting in a non-fluorescent substrate. Dual-labeled probes are designed to hybridize to a complementary region of the cDNA. The probe is flanked by an upstream and downstream primer pair that generates a PCR product. During PCR, when the polymerase extends the PCR product from the upstream primer, the 5' exonuclease activity of the polymerase cleaves the probe. This separates the fluorescent quencher and reporter dyes and Fluorescence Resonance Energy Transfer (FRET) no longer occurs. The increase in fluorescence intensity is proportionate to the number of probe molecules that are cleaved.



BioMark System for Genetic Analysis

The BioMark™ System includes the optical, thermal cycling, and software components necessary to perform real-time analysis of quantitative PCRs (qPCR) on Dynamic Array™ IFCs by Fluidigm.

The BioMark System, by Fluidigm, provides orders of magnitude higher throughput for real-time qPCR compared to conventional platforms due to its Dynamic Array™ IFCs—nanofluidic chips that contain fluidic networks that automatically combine sets of samples with sets of assays. This innovative solution for real-time qPCR provides experiment densities far beyond what is possible with microplate platforms, and significantly reduces the number of liquid-handling steps and volumes per reaction.

High-end Detection Optics

The BioMark System includes a 300-watt Xenon lamp and a 10-position excitation filter wheel (five wavelength positions and five emission filter positions). It also includes a high-resolution CCD camera that covers 30mm by 30mm, an area sufficiently large to simultaneously image all reactions in Dynamic Array™ IFCs. The BioMark System has optics as well as analysis software that are compatible with a variety of Fluidigm's chip families for TaqMan® chemistry. The BioMark System’s computer-controlled chip tray minimizes manual work. A barcode reader tracks experiments, reducing the chance of errors.

The BioMark System Components

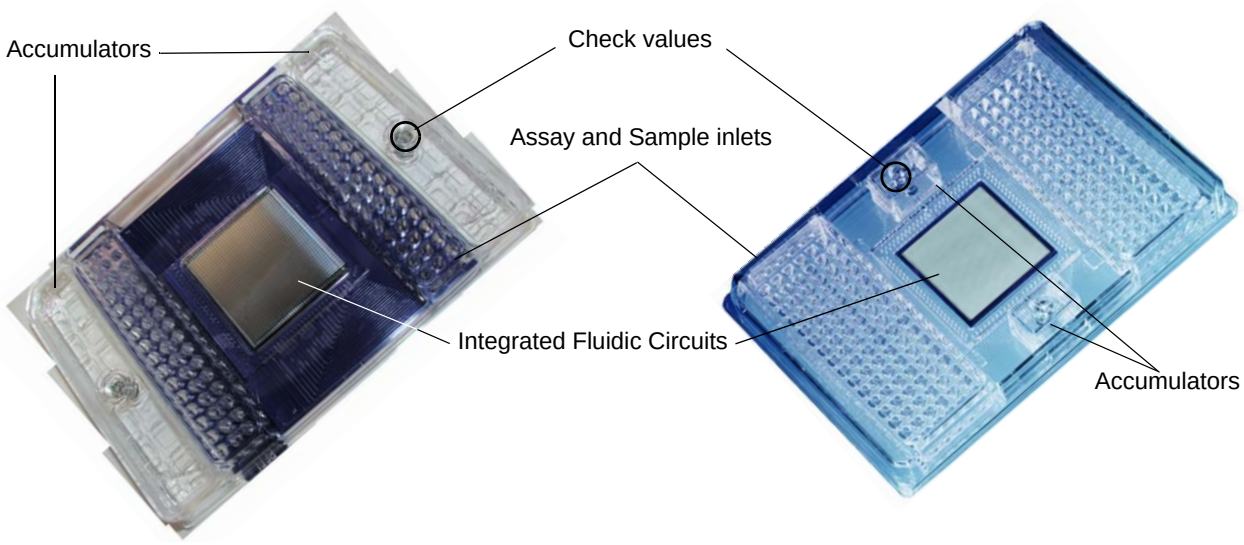
BioMark System including internal thermal cycler, flat panel monitor, keyboard, and mouse

NanoFlexController with laptop computer and handheld barcode scanner

The IFC Controller (single-bay). The MX model primes and loads 48.48 chips while the HX model primes and loads the 96.96 chips.

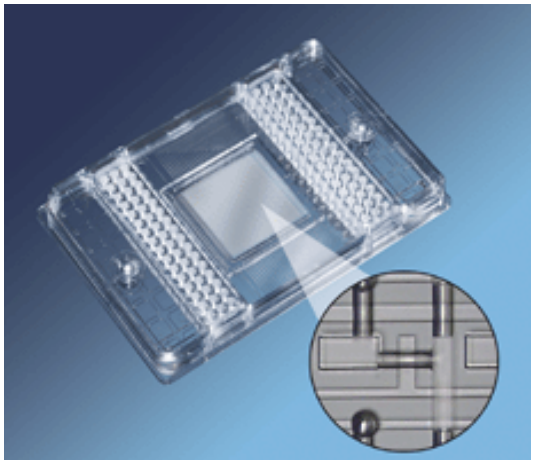
Dynamic Array™ IFC Components

Although chip architecture varies, the essential components common to all are highlighted in the graphic below. For more information see the appendices.



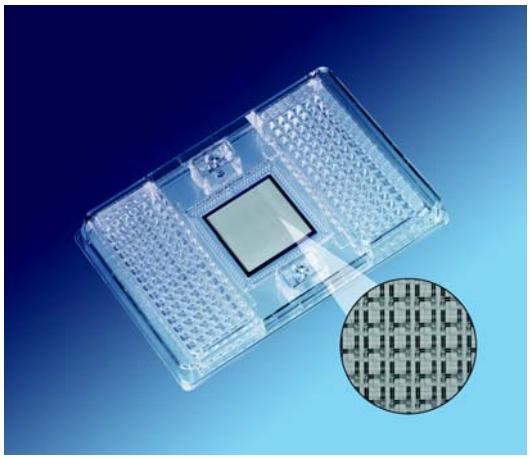
48.48 Dynamic Array™ IFC for Real-Time Quantitative PCR

The BioMark 48.48 Dynamic Array™ IFC is a matrix of channels, chambers, and integrated valves finely patterned into layers of silicone. Valves within the array partition 48 samples and 48 TaqMan® assays, and allow them to be systematically combined into 2,304 reactions.



96.96 Dynamic Array™ IFC for Real-Time Quantitative PCR

The BioMark 96.96 Dynamic Array™ IFC is a matrix of channels, chambers, and integrated valves finely patterned into layers of silicone. Valves within the array partition 96 samples and 96 TaqMan® assays, and allow them to be systematically combined into 9,216 reactions.



The following table illustrates the advantages of Dynamic Array™ IFCs, compared to microwell plates, using TaqMan® assays.

Dynamic Array™ IFC Features	Dynamic Array™ IFC Benefits
Lower running costs. Nanoliter reaction volumes	Saves on reagents and pipette tips, and on the upkeep of liquid-handling robots.
Higher throughput. High-density reaction chambers	The 48.48 Dynamic Array™ IFC provides 2,304 data points per run. The 96.96 Dynamic Array™ IFC provides 9,216 data points per run.
More Informative. An N x M (rows by columns) architecture coupled with nanoliter reaction volumes	Generates multiple readouts per sample, without the spectral overlap and cross-amplicon influence of multiplexed PCRs.
Highly Flexible. Input frame with microwells together with an N x M architecture	Facilitates the input of any set of samples and any set of primers/probes (detectors), delivering the throughput of a fixed array.

After pipetting samples and reagents into microwells on the chip frame, the chip is placed into the NanoFlex IFC (Integrated Fluidic Circuit) Controller (four bay) or the IFC Controllers (single bay) MX and HX. You use a laptop computer and a software user-interface to control the valves and pressure-load the sample and reagent fluids. Samples and reagents are automatically routed to their respective chambers for PCR amplification.

BioMark System Process Overview

The simplicity of running experiments on the BioMark system is illustrated in the five-step process below. More more information see, “48.48 Dynamic Array™ IFC Real-Time PCR Workflow” on page 131 and “96.96 Dynamic Array™ IFCs Real-Time PCR Workflow” on page 137

1. Prime the chip.
2. Add the samples and assays to the chip.
3. Load and mix samples and assays.
4. Run your real-time experiment on the BioMark system.

Before You Begin

To ensure good experiment results, follow the guidelines listed in the next four sections.

Organizing Your Work

- Label all reagent and reaction tubes.
- Maintain a separate DNA-free laminar flow hood—do not use nucleic acid samples in this hood.
- Use dedicated pipettes, tubes, and gloves for all manipulations that *do not involve* nucleic acid samples, which never leave the DNA-free (“Sample”) laminar flow hood.

Preventing Contamination

- Manipulate DNA samples under a dedicated laminar flow hood (name it, “Sample,” for example).
- Use separate dedicated pipettes, tubes, and gloves for all manipulations involving nucleic acid samples, which never leave the DNA-dedicated laminar flow hood.
- Change gloves frequently.
- Use aerosol-resistant disposable pipette tips. Discard tips after each use.
- Use disposable, UV-irradiated plastic ware.
- Ensure that all equipment, including paper, pens, and lab coats are dedicated for use only in a particular laboratory. For example, dedicated laboratory coats for each of the PCR rooms.
- Do not bring contaminated workbooks into clean PCR areas.
- Aliquot PCR reagents.
- Wipe PCR hoods daily with DNA*Zap*™ (Ambion) or a similar DNA decontaminate.
- Use ultra-violet radiation to complete decontamination.

- Ensure that only authorized users work in PCR areas and handle PCR equipment.
- Prepare reagents in a dedicated DNA-free laminar flow hood. DNA-free areas prohibit *any biological material***,** including DNA or RNA extracts, and PCR products. Also, in the DNA-free area, prepare and aliquot reagent stocks and reaction mixes.

Handling Nucleic Acid, PCR Mixes, and PCR Reactions

- Prevent carry-over of amplified DNA sequences by setting up PCR reactions in a dedicated laminar flow hood, while keeping post-PCR manipulations separate.
- Add extracted DNA to the PCR reaction mixes in the DNA-dedicated ("Sample") laminar flow hood. Be sure to prepare the PCR reaction mixes in the DNA-free laminar flow hood.
- Keep the amplification room—where PCR machines are housed—separate from the room in which PCR reactions are *assembled* (DNA free laminar flow hood).

Using Controls

- Include—whenever possible—a positive control that amplifies weakly but consistently. Using a strongly positive control sample may result in excess amplified product which may serve as a source of contamination.
- Use well-characterized negative samples such as lambda DNA.
- Include reagent controls containing all the necessary reagent components but excluding test DNA.
- Use decontaminating enzymes such as uracil N-glycosylase (UNG) or Uracil-DNA Glycosylase (UDG) to further minimize the likelihood of contamination.

What You Need for Experiments

This section describes the materials that you need to perform your experiments including reagents we support and sample requirements. In addition, you need the following:

- BioMark System
- NanoFlex Controller (4-bay) **or** IFC Controller (single bay)
- 48.48 Dynamic Array™ IFC **or** 96.96 Dynamic Array™ IFC
- 20X GE Sample Loading Reagent (Fluidigm PN 85000735)—store at 4ºC.
- 2X Assay Loading Reagent (Fluidigm PN 85000736)—store at 4ºC.
- Deionized DNA-free, DNase-free, RNase-free water—store at room temperature.
- TE buffer: 10 mM Tris-HCl, 0.1 mM EDTA, ph 8.0 (Technova PN T0221)—store at room temperature.
- Sample Mix (see "Preparing Sample Pre-Mix and Samples" on page 133).
- Prime/probes sets

- Samples of interest

Supported Detection Reagents

We support the following detection reagents[1] with the BioMark System.

Probe Types

- FAM-MGB
- VIC-MGB
- FAM-TAMRA
- FAM-non fluorescent quencher[2]

Additional Probe Types

Fluidigm does not support other probe types at this time, however, additional probe types may be run with the BioMark System using the following guidelines:

Fluorophores With...		
Excitation Wavelengths		Emission Wavelengths
between 465 and 505 nm	And	between 500 and 550 nm
between 510 and 550 nm	And	between 540 and 600 nm

PCR Master Mixes

The protocol described in this manual uses TaqMan® Universal PCR Master Mix (2X) (Applied Biosystems, PN 4304437). If you choose to use master mixes other than TaqMan® Universal PCR Master Mix, you may have to alter the protocol described in this manual. Contact Fluidigm Technical Support for additional information.

IMPORTANT: You must use a passive reference.

1. Fluidigm recommends that you only use TaqMan® probes and/or other licensed PCR assay reagents from authorized sources. If you have any questions regarding whether you have a license to use particular reagents in PCR systems, you should contact the appropriate licensor and obtain clarification and their permission if necessary. For example, certain probes and their use may be covered by one or more patents held by Applied Biosystems and/or Roche Molecular Systems, which may be contacted at the Director of Licensing at Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404 or the Licensing Department, Roche Molecular Systems, Inc., 1145 Atlantic Avenue, Alameda, California 94501.
2. Contact Fluidigm technical support to discuss your non-fluorescent quencher requirements.

Sample Requirements

DNA Quality

Your cDNA should have an 260:280 Ratio between 1.5 and 1.8. Prior to use on a chip, monitor the integrity of your cDNA on a system such as the Agilent® 2100 bioanalyzer.

cDNA Input

The exact amount of cDNA to be used for each experiment depends on the relative abundance of the target gene. Unless you have concentrations in excess of 1,000 copies of your target template per µl of sample, we recommend that you increase the your target concentration by using target specific amplification as described in Chapter 4, “Multiplex Target Specific Amplification Protocol for Gene Expression Analysis,” in the *BioMark Real-Time Quantitative PCR Data Collection User Guide (PN 68000080)*.

cDNA Storage

Avoid multiple freeze-thaw cycles by storing cDNA at 4ºC. For longer storage, aliquots may be stored at -20ºC.

Using Real-Time PCR Analysis Software 2

In this chapter

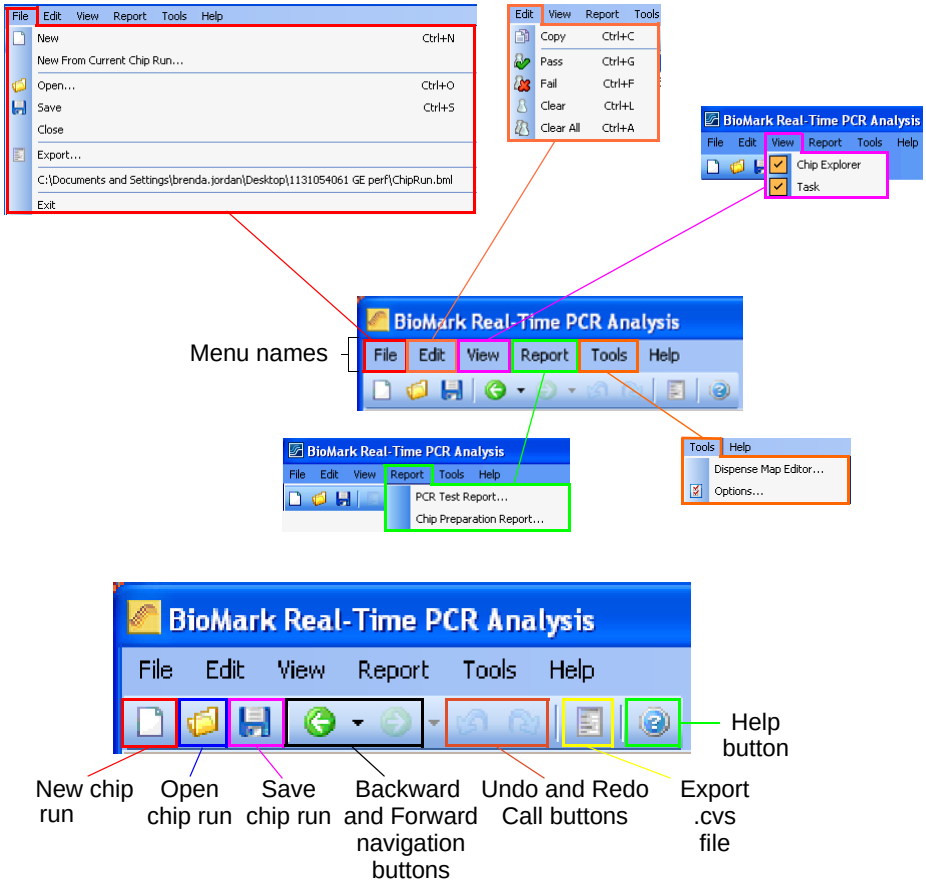
Launching the Software

1 Double-click the BioMark Real-Time PCR Analysis software icon on your desktop to launch the program.

Then Start Window opens, as shown below.

2 Click the maximize button for optimum viewing.

Menu Bar



NOTE: You can use the Call Redo or Undo buttons to revert back to the original call state.

Menu Bar Options

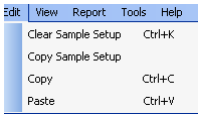
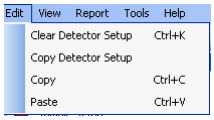
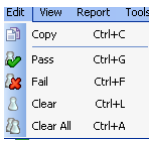
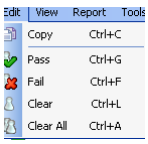
File

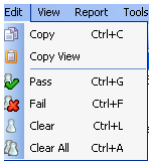
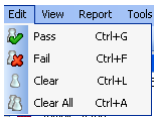
The File menu names are described below.

Menu Name	Description
New	Opens the Chip Run Setup Wizard
Open	Opens the location of .bml chip run data files
Save	Saves your current run data file with any changes
Close	Closes your current run data file
Export	Exports Results table data or Heat Map data as .csv text file
C:\...	Location of recently viewed/used .bml files
Exit	Closes the BioMark Real-Time PCR Analysis software application

Edit

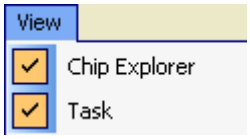
The Edit menu options are dependent on the active window.

If the Active Window is...	Then Your Options Are...
Sample Setup	
Detector Setup	
Analysis Views	
Results Table	

If the Active Window is...	Then Your Options Are...
Image View	
Heat Map	

View

Click a pane name to collapse or expand it.



Report

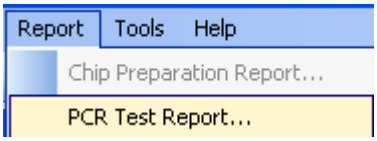
Two reports are available: PCR Test and Chip Preparation.

PCR Test Report

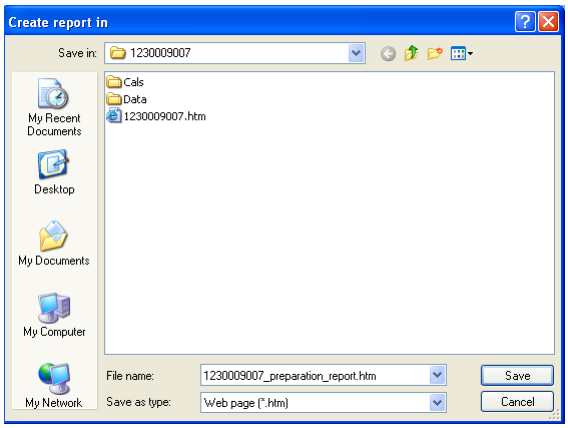
The PCR Test report is only available after a chip run (.bml) file has been opened and analyzed in the software.

1 Click **Report**.

2 Click **PCR Test Report**.



The Create File In dialog box opens.



3 Click an existing folder, create a new folder, or navigate to a folder where you want to save.

4 Click **Open**.

5 Click **Save**. The chip run information is saved as an .htm file (web page). It opens automatically in your default browser after you click Save.

BIOMARK™
GENETIC ANALYSIS BY FLUIDIGM®

PCR Test Report

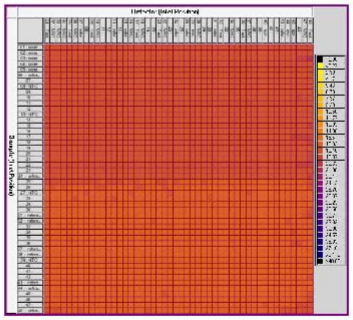
Summary

Barcode:	1130085012
Chip Type:	48.48
File Location:	C:\chipruns\1130085012\ChipRun.bml
Protocol:	Default-10min-Hot-Start
Passive Reference:	ROX
Probe Type(s):	FAM-MGB
Experiment Start Time:	1/15/2007 3:05:55 PM
Duration:	01:39:44
System ID:	ALPHA003
Comment:	Gene Expression

Heat Map

Source:
FAM-MGB Ct

View Type:
Inlet-based View

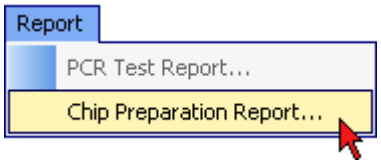


PCR Test Result

	Entire Chip:		
	Mean Ct	Std. Dev.	% No Call
Sample 2X	17.17	0.09	0.00
Sample 1X	16.11	0.09	0.00
Test Result	1.06	0.09	0.00

Chip Preparation Report

The Chip Preparation report records the loading pattern for a chip run. After creating a new chip run file, use the Chip Preparation report to record the data for hand-pipetting. Click **File > Save As** to save the .htm file. Print the report for convenient access.



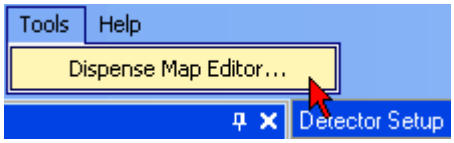
NOTE: One .htm page is created for each page in the chip preparation report.


BIOMARK
GENETIC ANALYSIS BY FLUIDIGM
Chip Preparation Report
Summary
Barcode:
Chip Type: M48.48
File Location: C:\Documents and Settings\michael.diederich\Desktop\test\ChipRun.bml
Protocol:
Passive Reference: ROX
Probe Type(s): Test
Comment:
Sample Setup
Source: 96 Wellplate
Name:
Barcode:
Mapping: M48-Sample-SBS96-Left
Click here to enlarge.
Sample Mapping


Click here to enlarge.
Detector Setup
Source: 96 Wellplate
Name:
Barcode:
Mapping: M48-Detector-SBS96-Left
Probe Type: Test
Click here to enlarge.
Detector Mapping
Click here to enlarge.

Tools

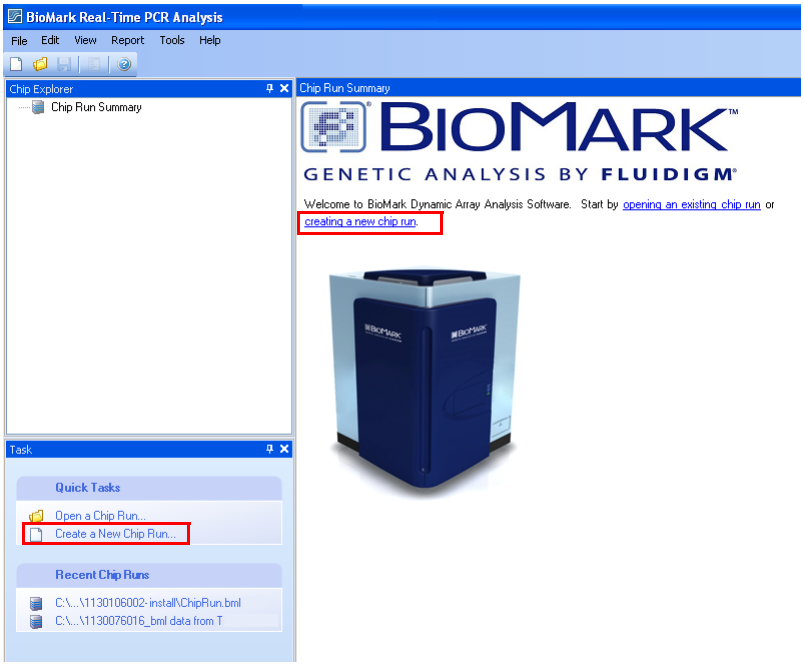
Access the Dispense Map Editor from this tool bar menu.



NOTE: For more information see, "Using the Dispense Map Editor" on page 41.

Creating a New Chip Run

1 Click **creating a new chip run** in the Chip Run Summary pane or, click **Create a New Chip Run** in the Task pane.



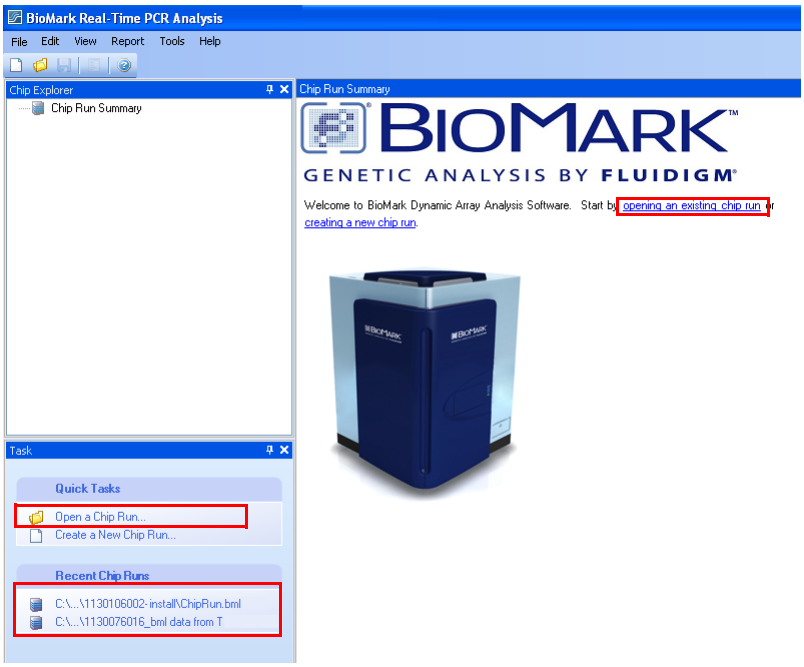
The Chip Run Setup Wizard opens.

2 Follow the steps at the top of the wizard to complete the setup.

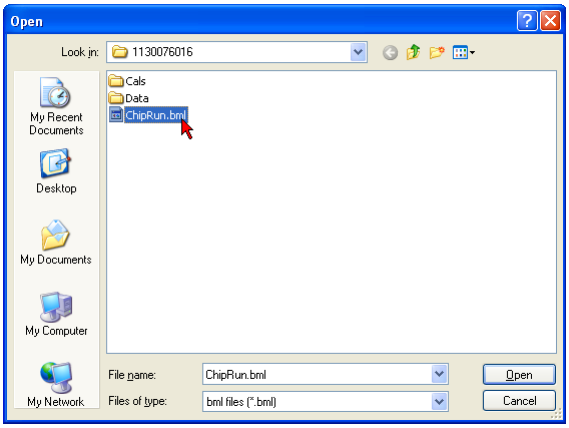
3 Complete the wizard and then proceed to "Setting Up a Sample Plate" on page 33.

Opening an Existing Chip Run

1 Click **opening an existing chip run** in the Chip Run Summary pane or, click **Open a Chip Run** in the Task pane or, **File > Open**.



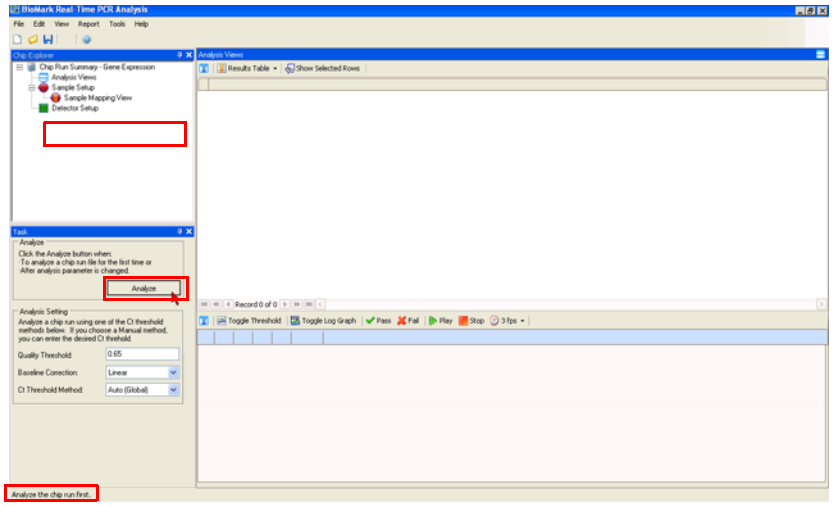
2 Double-click the chip run file (.bml extension).



A progress dialog box opens.

The chip run file opens in the Chip Explorer pane.

3 Click **Analysis Views**.

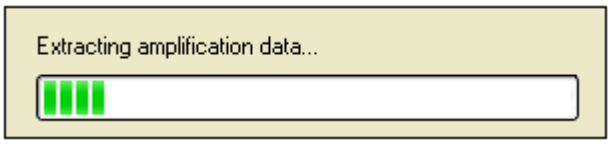


4 Click **Analyze**.

NOTE: A reminder to analyze the chip run appears on the lower left status bar.

IMPORTANT: You must click **Analyze** in the task pane each time you change parameters. A reminder dialog box opens, as well as a reminder in red text, if you fail to click Analyze after each change.

The Extracting dialog box indicates the start of chip analysis.

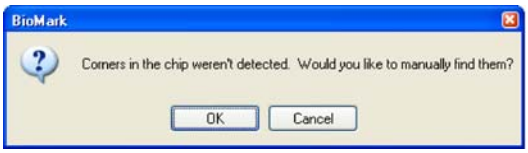


The first time a chip is analyzed, the chamber-finding algorithm locates the chamber boundaries of each captured image. Thus, this is the most lengthy step in the entire analysis procedure.

Continue with “Setting Up a Sample Plate” on page 33.

Finding Corners Manually

During the first analysis, if the chamber finding algorithm cannot locate the four corner cells of the chip, the following error message will appear:



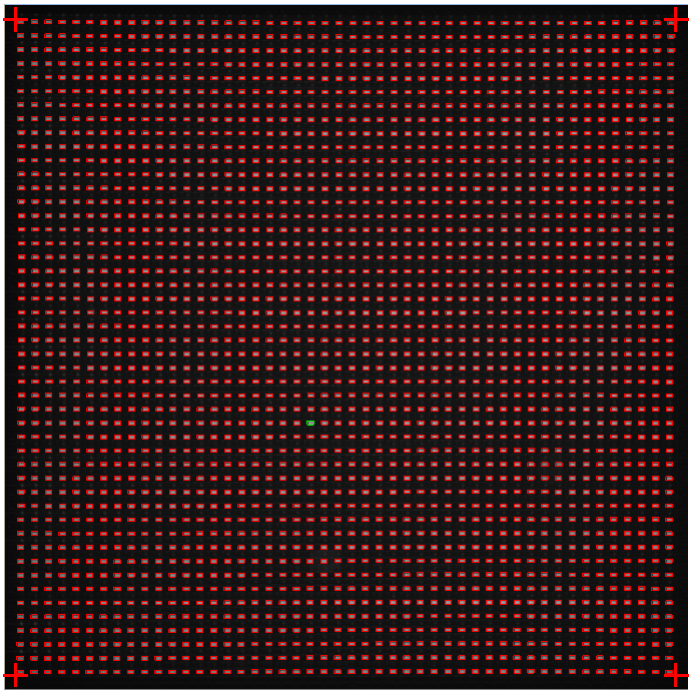
If this occurs, you can manually set the corners and then analyze the chip.

1 Click **OK** on the dialog box.

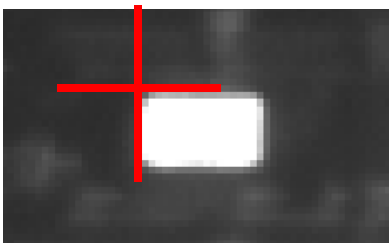
2 Zoom in to see the corner cells.

NOTE: If you cannot see the four corner cells, adjust the Contrast slider. (If an insufficient amount of ROX dye was used in setting up the chip corners, it will be difficult to see the corner cells.)

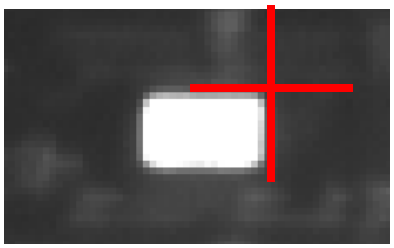
3 Move the red cross hairs to each of the four corner cells. Make sure each cross hair is on the outer edges of each corner cell (see below).

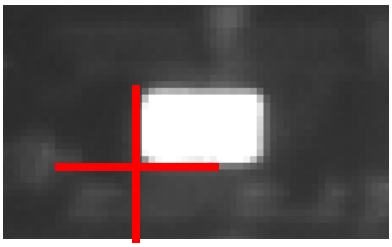


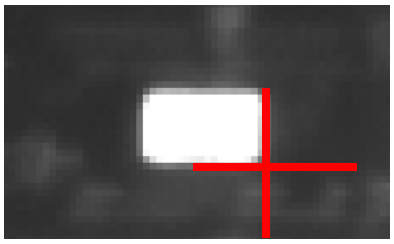
Upper left corner



Upper right corner





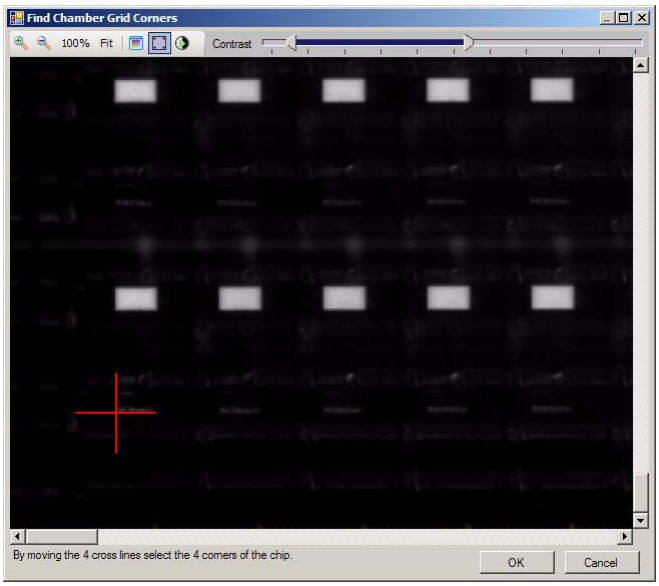


Lower left corner

Lower right corner

4 Click **Done**.

NOTE: If little or no ROX is present, the corner cells are very dark. You may have to count the number of rows and columns (48 down, 48 across) to make sure you are placing cross hairs correctly. See figure below.



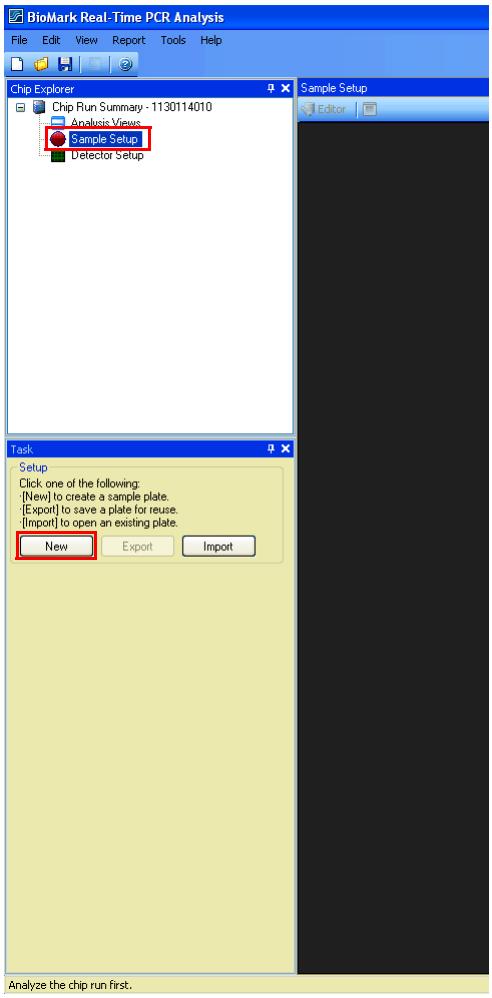
Forced Manual Corner Find

If the automated manual corner find results are not satisfactory, you can perform a forced manual corner find by pressing the **Ctrl** key and simultaneously clicking on the **Analyze** button.

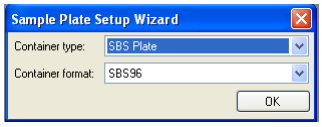
Setting Up a Sample Plate

Use the table below as a guide when choosing samples.

Sample Name	Description
 Blank	An unused position. Nothing in the chamber.
 NAC	**No Amplification Control:** usually the Taq polymerase is left out of the reaction; this is a negative control that confirms that positives cannot occur without the PCR working.
 NTC	**No Template Control (negative control)**: everything included except the sample; to show that a positive result cannot be obtained when the sample is left out.
 Unknown	An experimental sample.
 Reference	A sample against which the unknown samples are compared or normalized.
 Standard	A sample against which unknown samples are compared in a standard curve analysis.



The Sample Plate Setup Wizard dialog box opens.



1 Choose the appropriate Container type and Container format from the drop-down menus, using the table below as a guide.

Container type choices

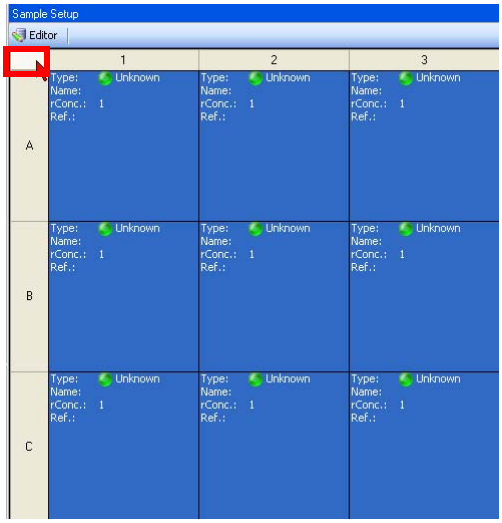
Container format choices

Drop-down Menu Item	Description
Container type	• **SBS Plate:** represents the plates where samples and detectors are stored before being pipetted into a chip. • **Sample Inlets**: location where samples enter the chip.
Container format	• SBS96: represents a 96-well plate.

2 Click **OK**.

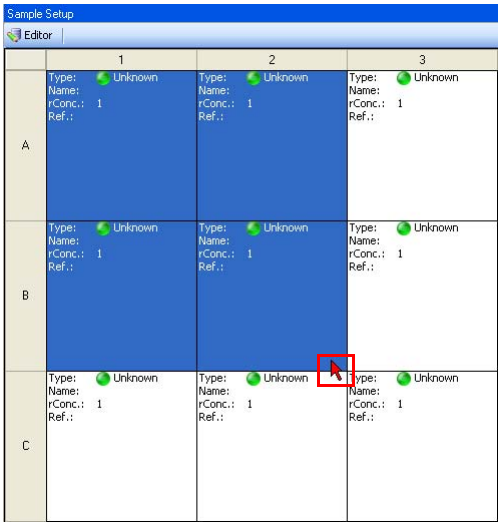
3 Select the cells you are going to use as a reference.

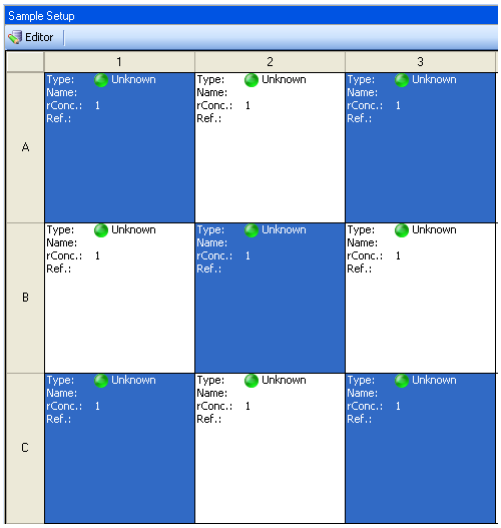
- Click the upper left corner to select all the cells.
- Click and hold while dragging your cursor through cells.
- Click individual cells while pressing the Ctrl key.



Click the upper left corner to select all cells

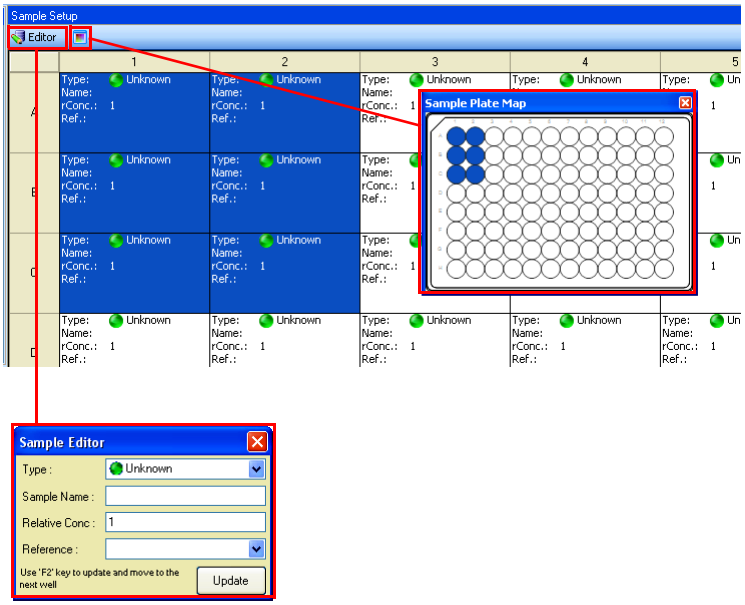
Click and drag to select



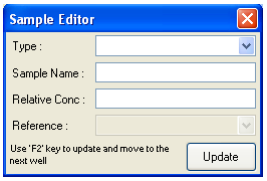


Press and hold the Ctrl key while clicking individual cells

4 (Optional) Click the Sample Plate Map icon .
The map shows selected cell(s) relative to the entire sample plate.

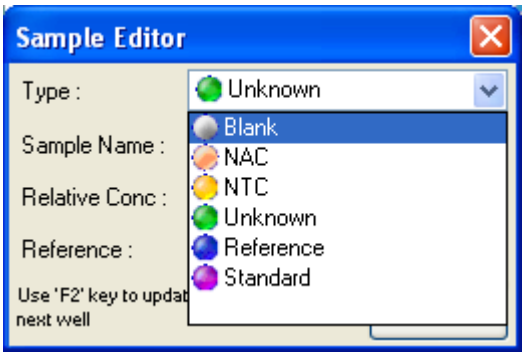


5 Click **Editor**.
The Sample Editor dialog box opens.



6 Select the appropriate type from the Sample Type drop-down menu.

NOTE: If you want to identify a reference before moving on, see "Calculating Delta Ct Sample Values" on page 110.

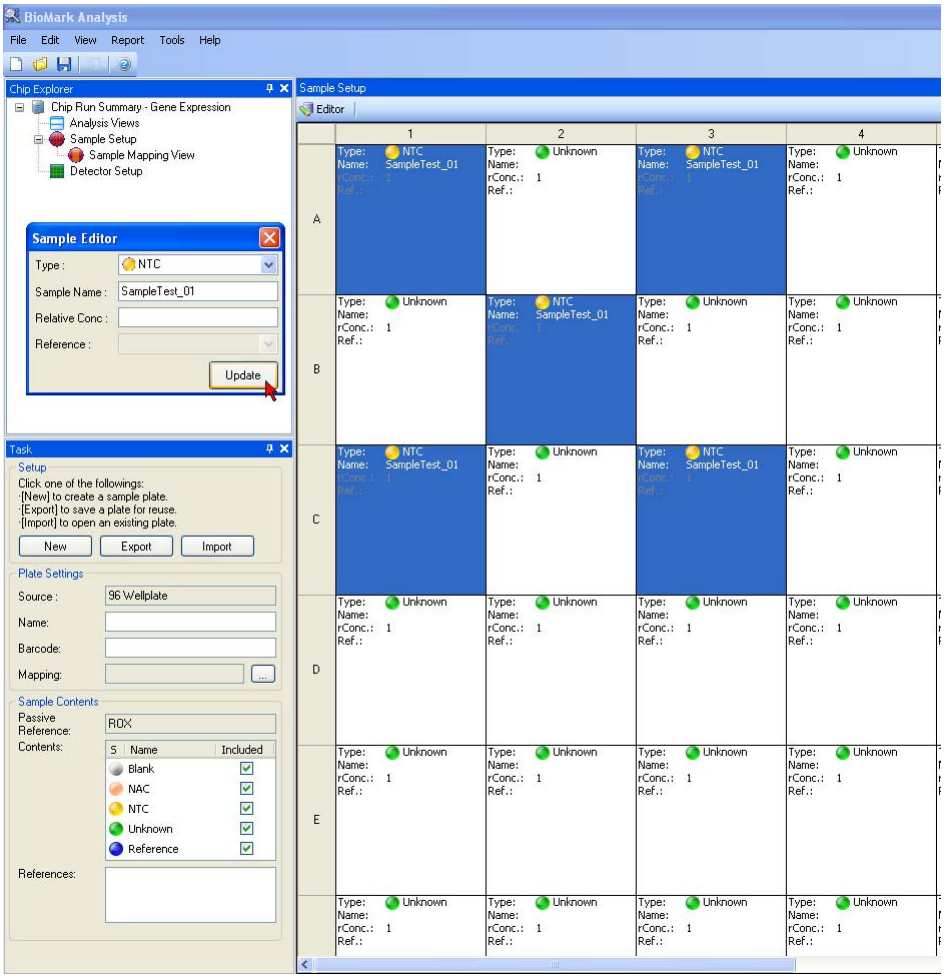


7 Enter the sample name.

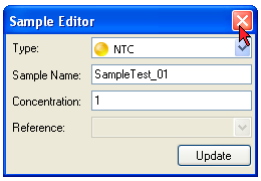
8 Enter the relative concentration.

9 Click **Update**.

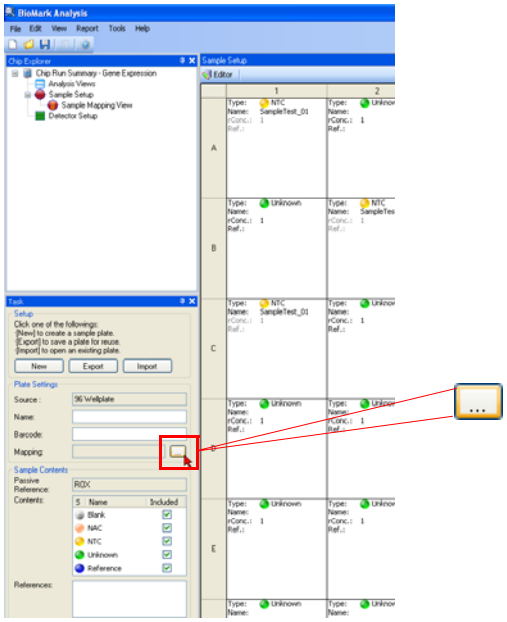
The Sample Plate Setup window now reflects the updates.



10 Close the Sample Editor.

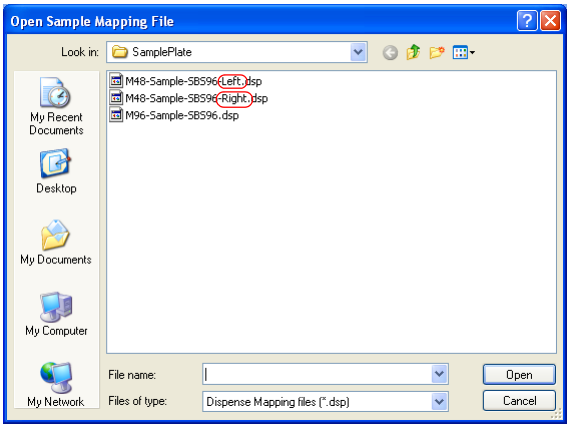


11 Click the Open Mapping File icon

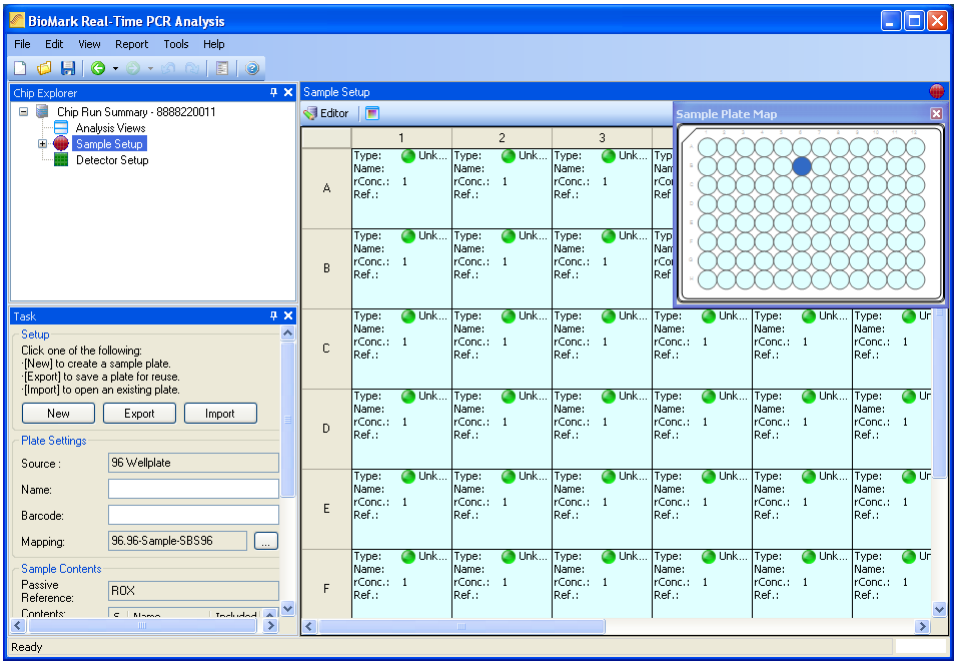


12 Double-click either left or right sample mapping file to determine dispense location.

NOTE: If you are analyzing a 96.96 chip, select M96-*Sample-SBS96.dsp*.



Your selection is displayed in light blue (left or right).

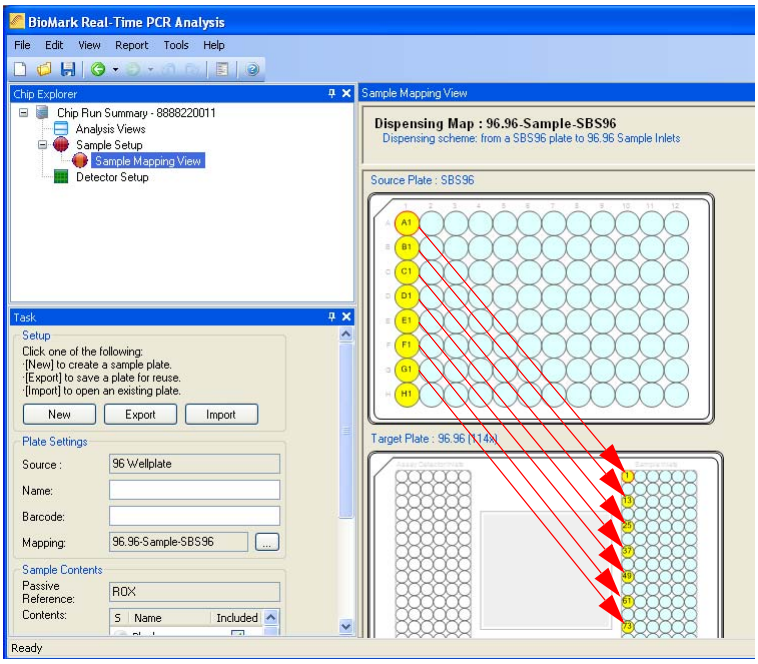


Your sample plate setup is complete. Proceed to the next section for Sample Plate Mapping.

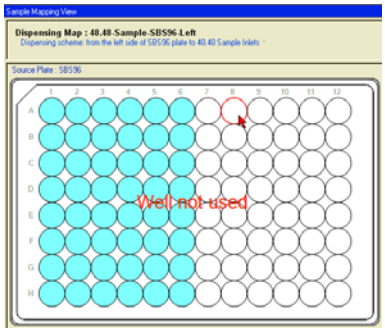
Using the Sample Mapping Viewer

After setting up the sample plate, view and/or record the loading pattern in the Sample Mapping Viewer.

1. Click Sample Mapping View in the chip explorer pane. The dispensing map opens.
2. Click a cell in the Source Plate to see where it loads on the Target Plate (see graphic below.)

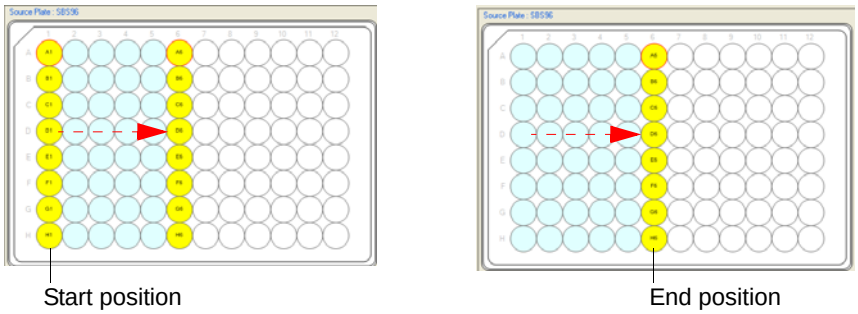


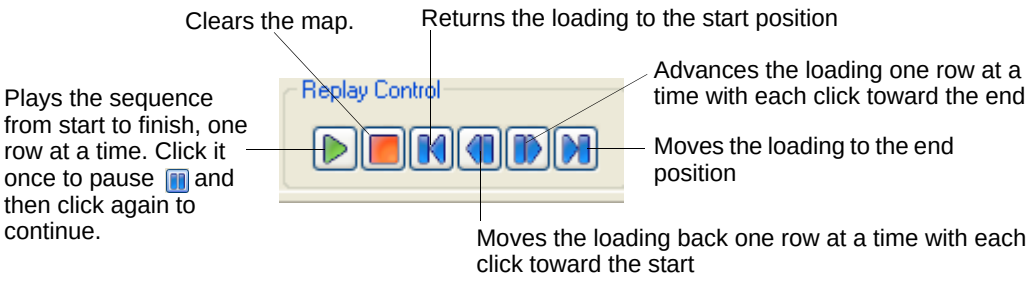
NOTE: If you attempt to click an unused cell, a warning appears:



Using the Replay Control

Use the replay controls to show you where and in what sequence the Target Plate receives the samples from the Source Plate.

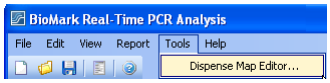




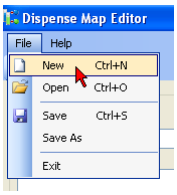
Using the Dispense Map Editor

Use the Dispense Map Editor to record custom load maps for future use. After recording your loading sequence, you can save it and play it back anytime.

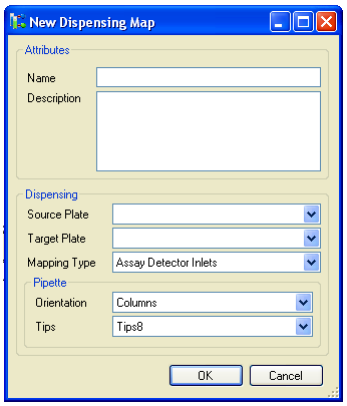
1 Click **Tools > Dispense Map Editor**.



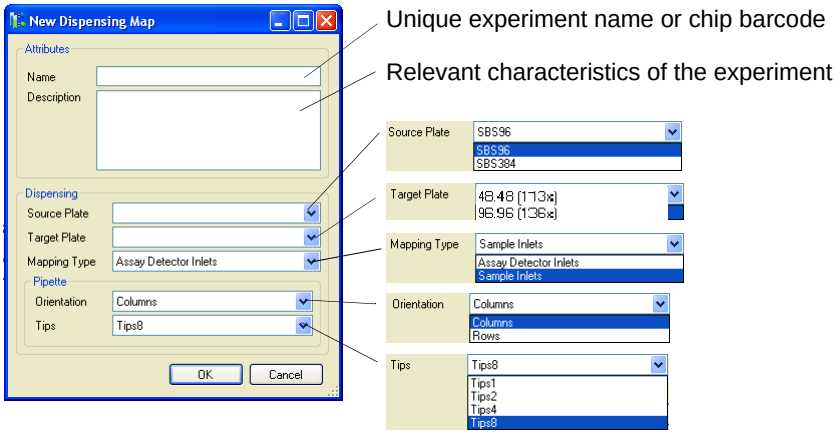
2 Click **New**.



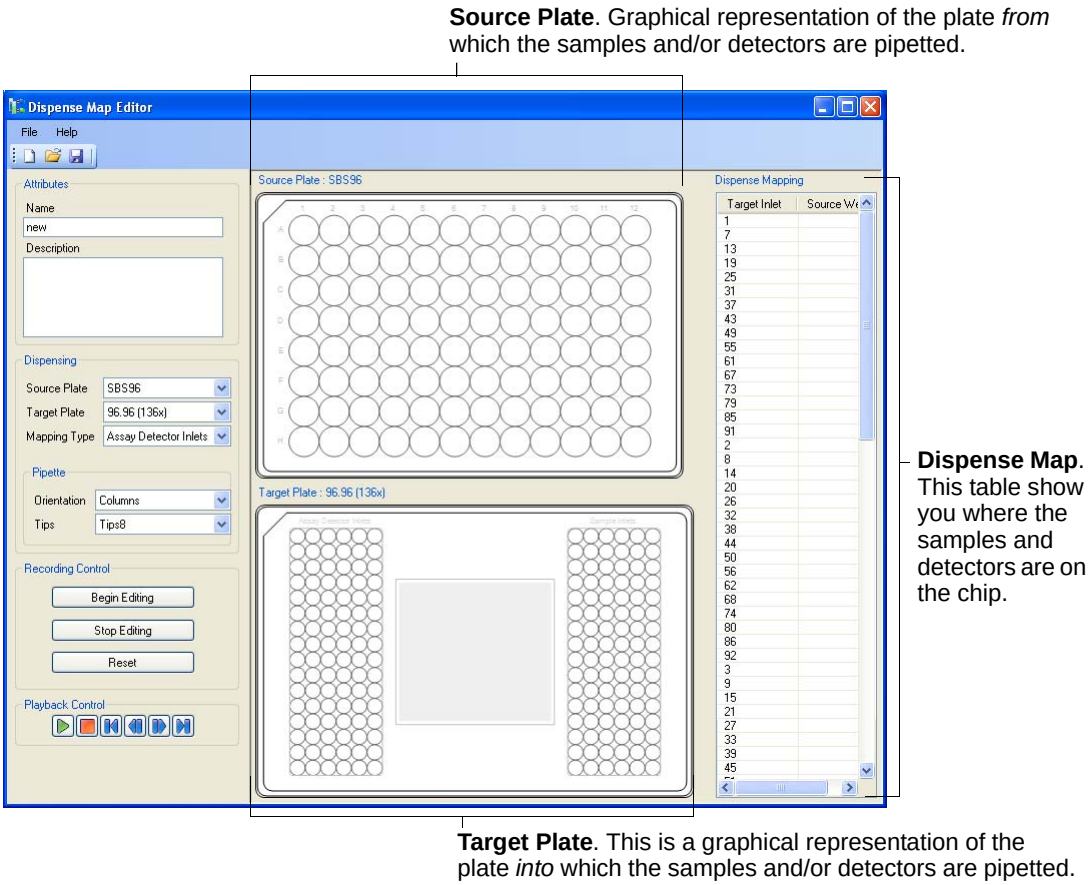
The New Dispense Map dialog box opens.



3 Complete the New Dispense Map dialog box using the following example as a guide.



4 Click **OK** to open the new dispense map in the Dispense Map Editor.



5 Click **Begin Editing** in the recording control pane.

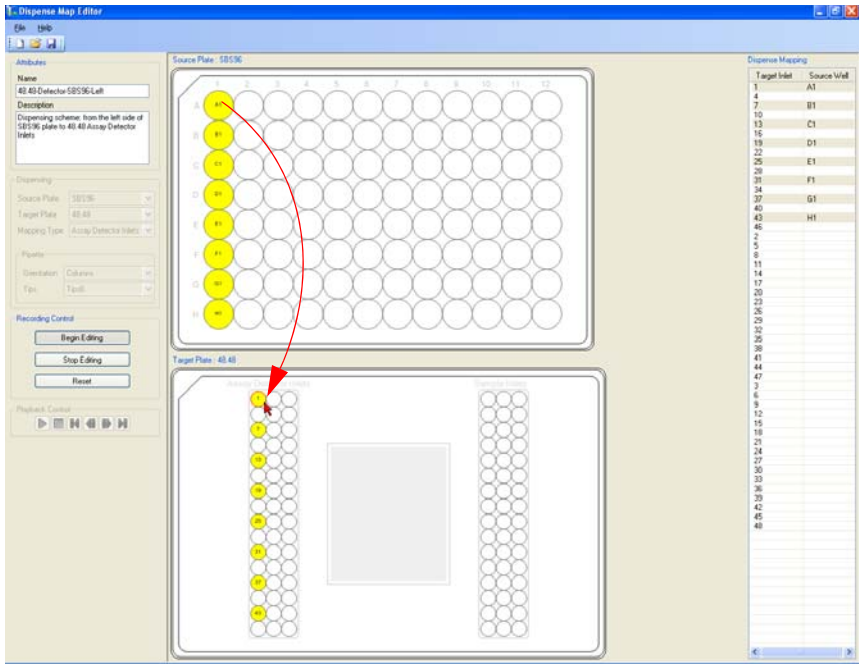
a Click the first cell from the Source Plate. Then, click the location in the Target Plate (see the figure in "Using the Sample Mapping Viewer" on page 39).

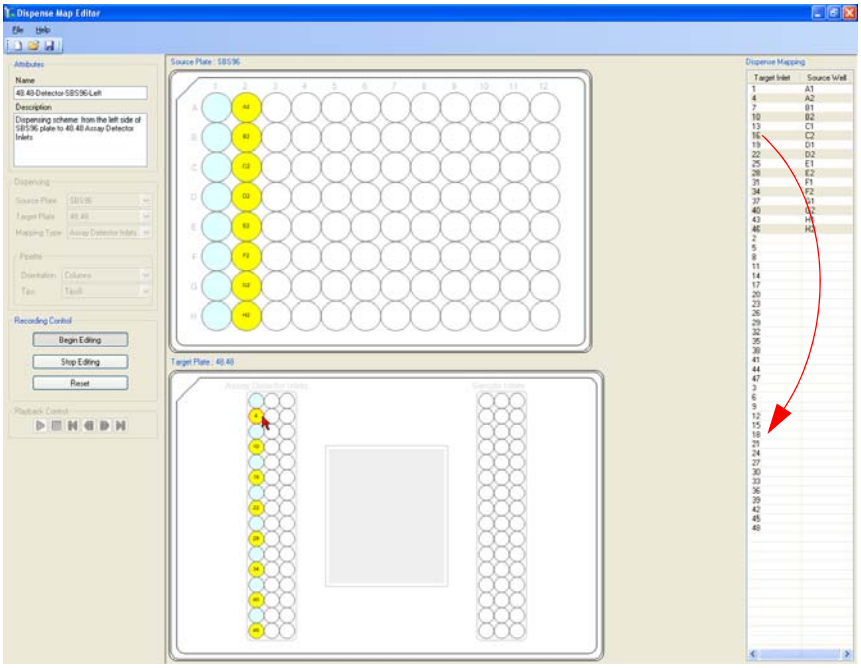
b Continue clicking appropriate cells (that is to say, from the Source Plate to the Target Plate) until your custom loading map has been recorded.

NOTE: When you click Begin Editing, the dispensing pane becomes inactive.

6 Click **Stop Editing**.

Refer to the following two graphics as an example of custom loading and how it looks as you proceed.





7 Review the loading pattern you have recorded by clicking the green arrow button in the playback control pane.

Setting up a Detector Plate

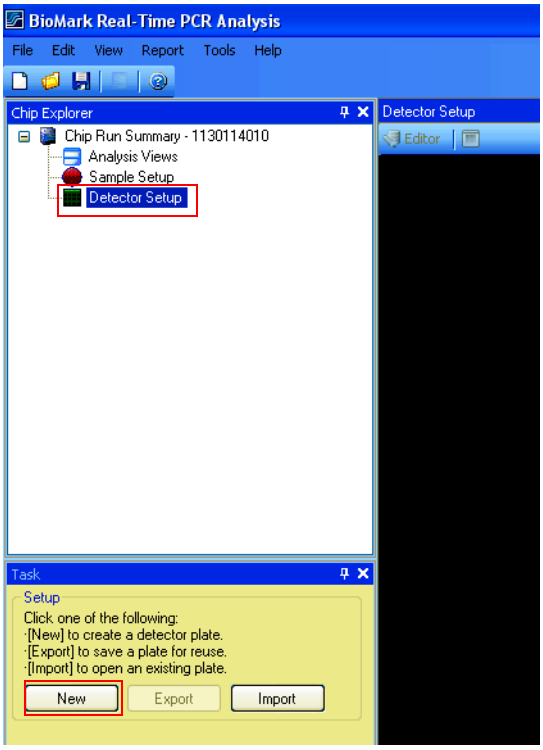
Use the table below as a guide when choosing detectors.

Detector Name	When to Choose
Test	Experiment reagents
Reference	A control or reference gene
NRC	No Reagent Control: negative control using only buffer, no primers/probes (detectors)

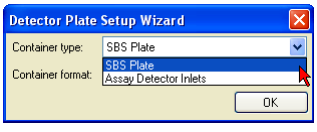
To Set Up the Detector:

1 Click **Detector Setup** to highlight.

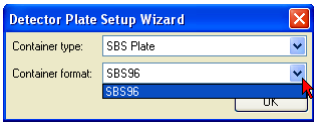
2 Click **New**.



3 Choose the appropriate Container type and Container format from the drop-down menus.



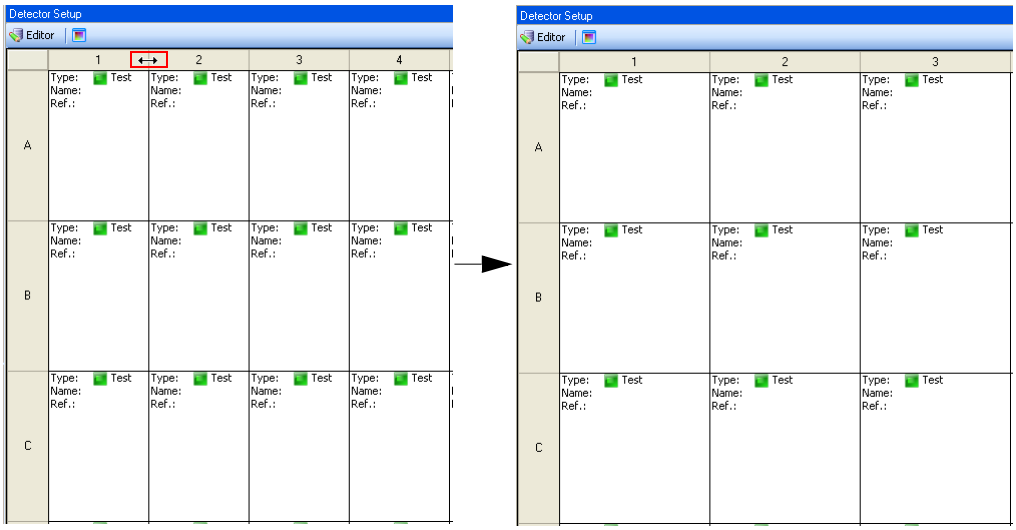
Container type



Container format

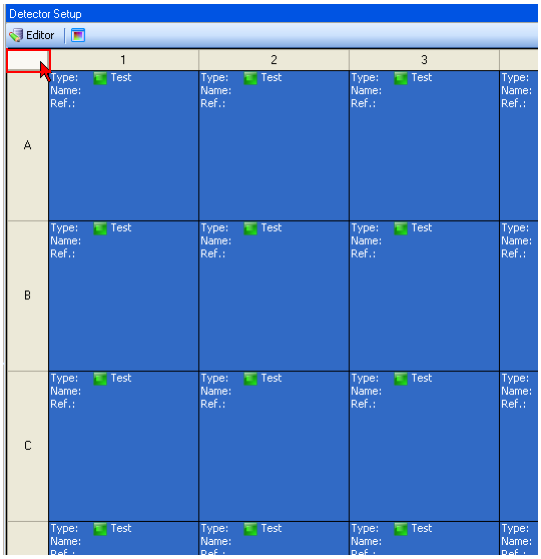
4 Click **OK** to open the Detector Plate window.

5 (Optional) Double-click between columns to expand them.

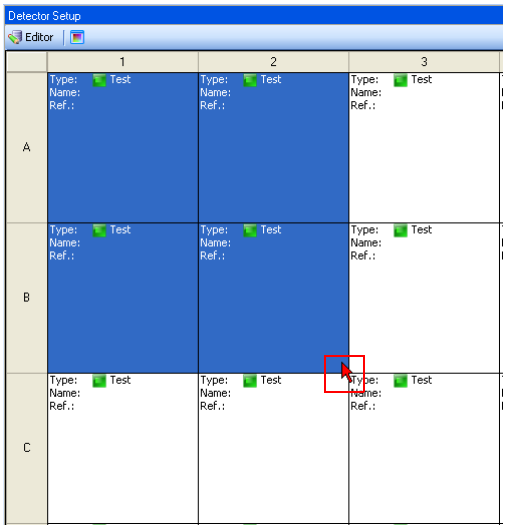


6 Select cells by performing one of the following:

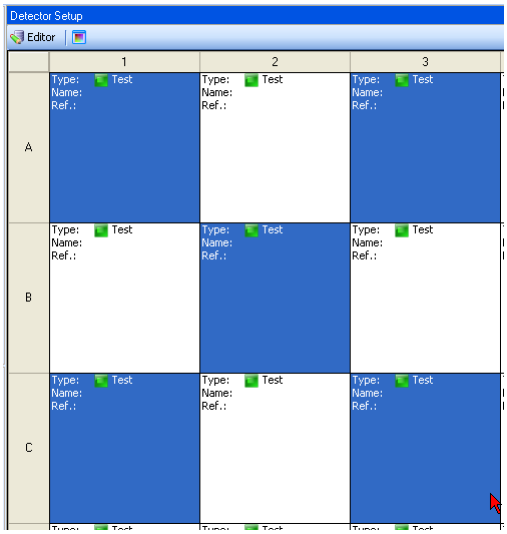
- Click and hold while dragging your cursor through cells.
- Click the upper left corner to select all the cells.
- Click individual cells while pressing the Ctrl key.



Click the upper left corner to select all cells



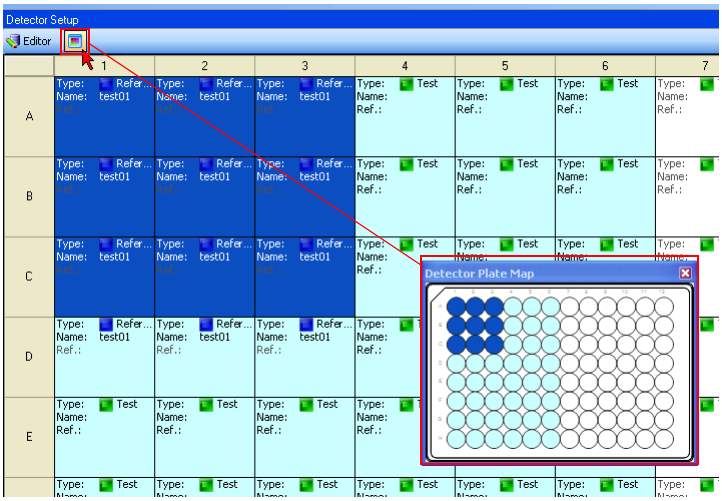
Click and drag to select



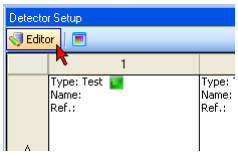
Press and hold the Ctrl key while clicking individual cells

7 (Optional) Click the Detector Plate Map icon .

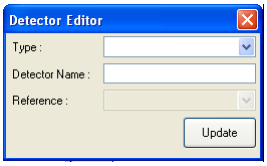
The map opens and shows selected cell(s) relative to the entire detector plate.



8 Click **Editor**.



The Detector Editor dialog box opens.

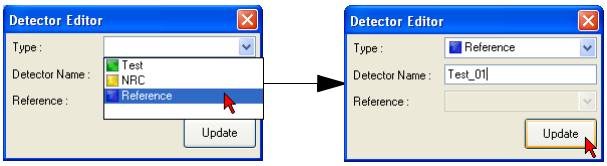


9 Complete the Detector Editor:

a Select the appropriate type from the Detector Type drop-down menu.

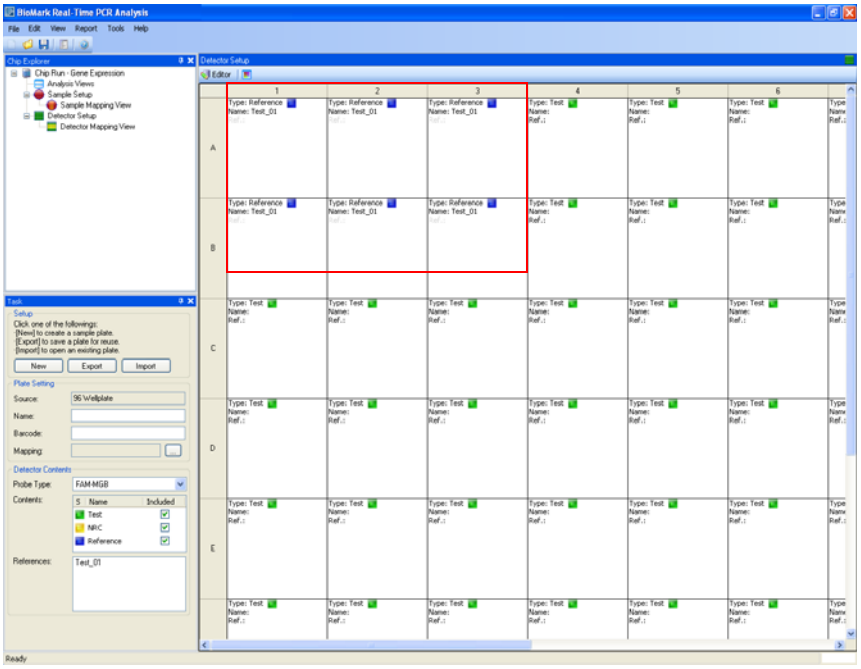
NOTE: If you want to identify a reference before moving on, see "Calculating Delta Ct Detector Values" on page 114.

b Enter the Detector name.

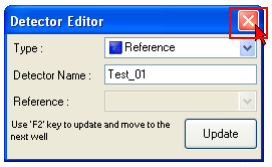


10 Click **Update**.

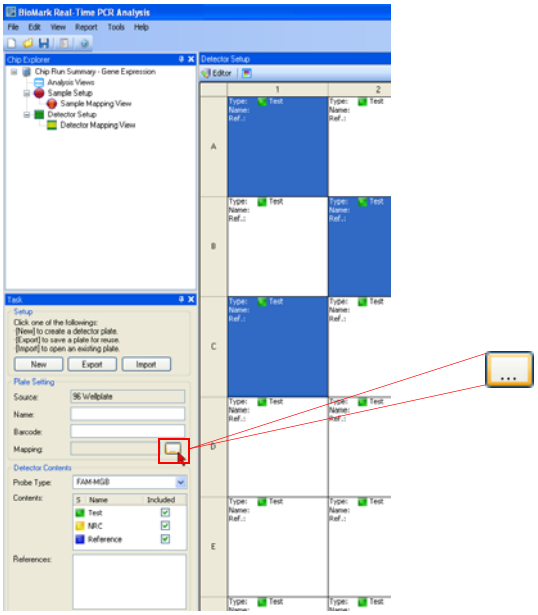
The Detector Plate Setup window now reflects the updates.



11 Close the Detector Editor.

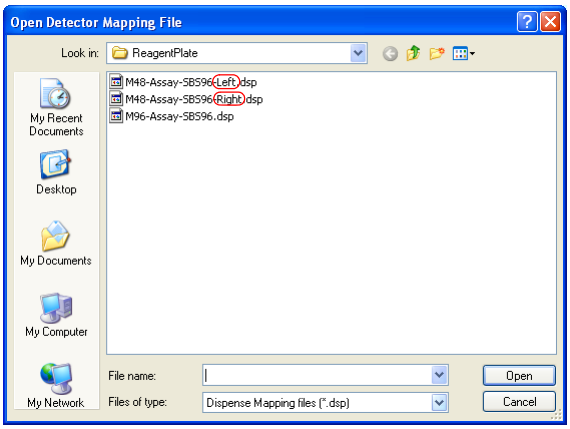


12 Click the Open Mapping File icon.

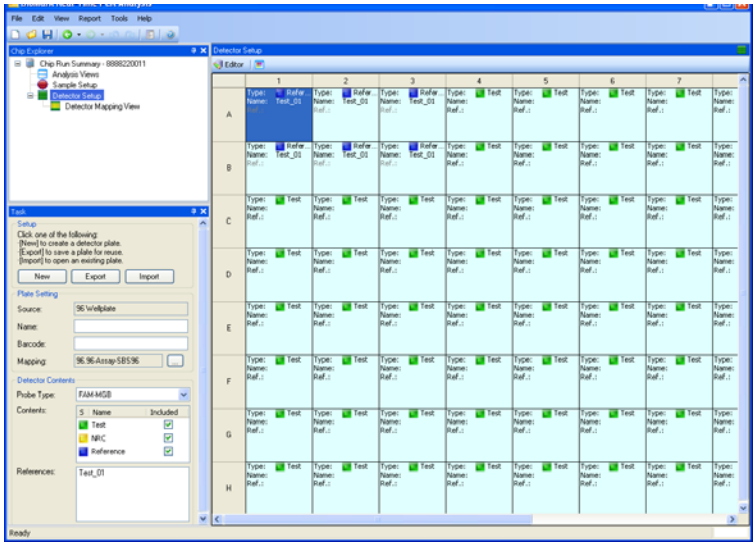


13 Double-click either left or right sample mapping file.

NOTE: If you are analyzing a 96.96 chip, select *M96-Assay-SBS96.dsp*.



Your selection is displayed in light blue (left or right) in the Mapping Viewer (graphic below).



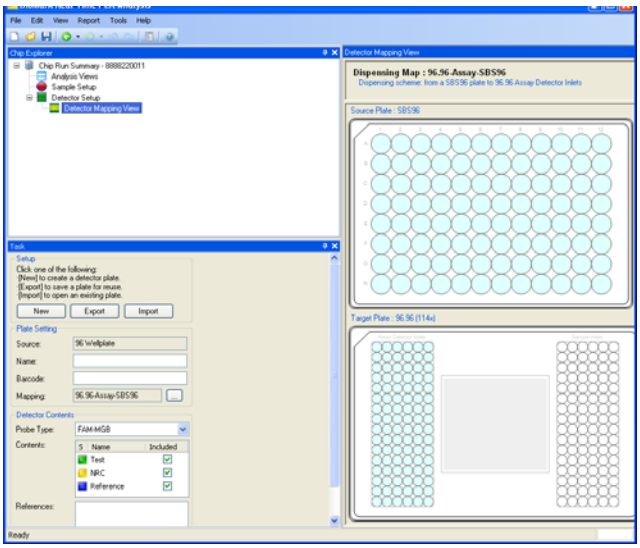
Your detector plate setup is complete. The next section shows you how to use the Mapping Viewer.

NOTE: You can also copy and paste sample/assay names directly from Microsoft® Excel sheets.

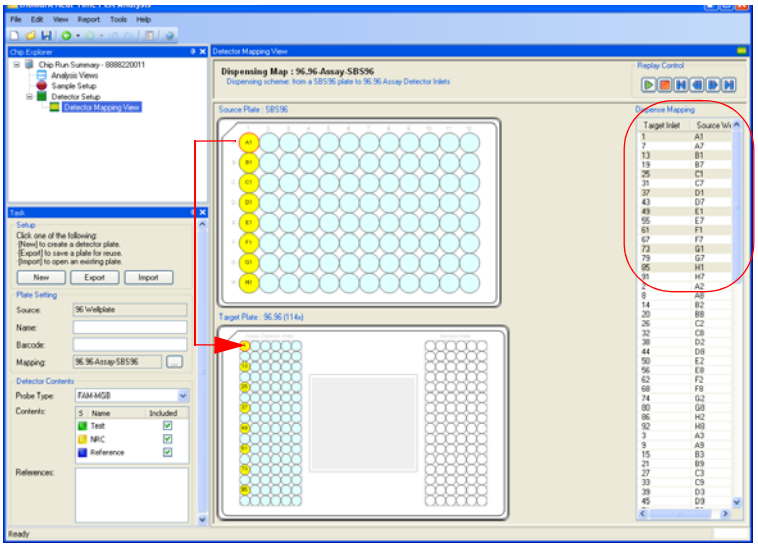
Using the Detector Mapping Viewer

After setting up the detector plate, view and/or record the loading pattern in the Detector Mapping Viewer.

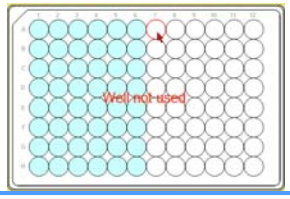
1 Click **Detector Mapping View** in the chip explorer pane. The dispensing map opens.



2 Click a cell in the Source Plate to see where it loads on the Target Plate (see the following example).



NOTE: If you attempt to click an unused cell, you get a warning:

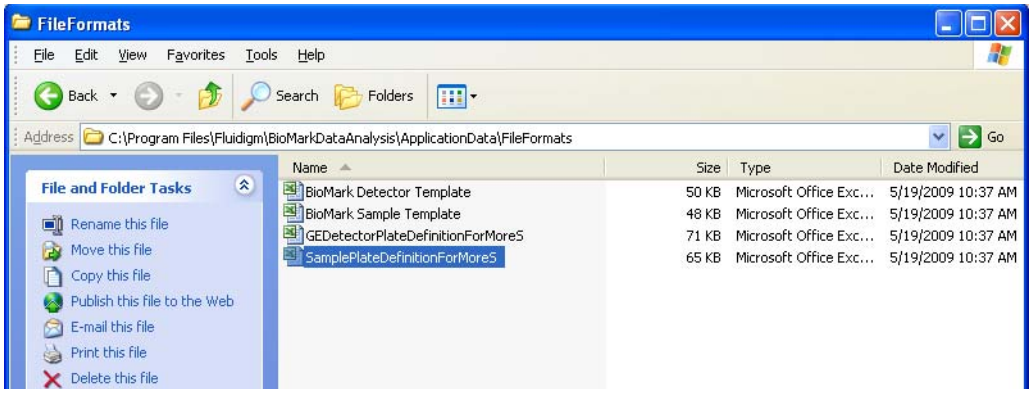


Converting a Chip Run to a More Samples Run

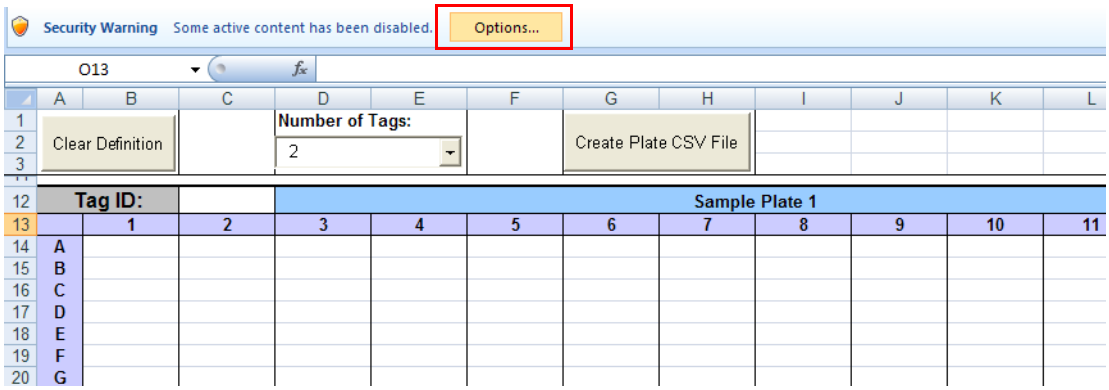
The More Samples feature requires special sample and detector setup. Fluidigm provides setup templates in the Microsoft Excel® file type. You can use the following workflow to set up your samples and detectors and convert them to .csv files and then templates in the analysis software.

Sample Setup

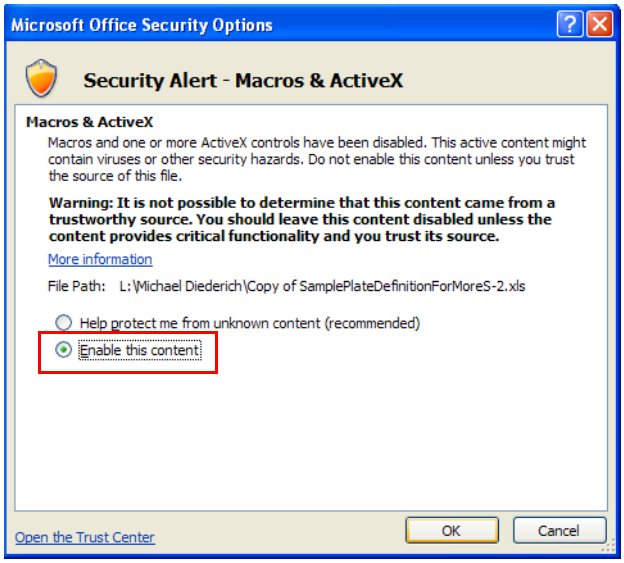
1. On your BioMark or EP1 system computer, go to *C:\Program Files\Fluidigm\BioMarkGenotypingAnalysis\ApplicationData\FileFormats*.
2. Open the file labeled ***SamplePlateDefinitionForMoreS***.



3. Click **Options** to enable Active X (if prompted).



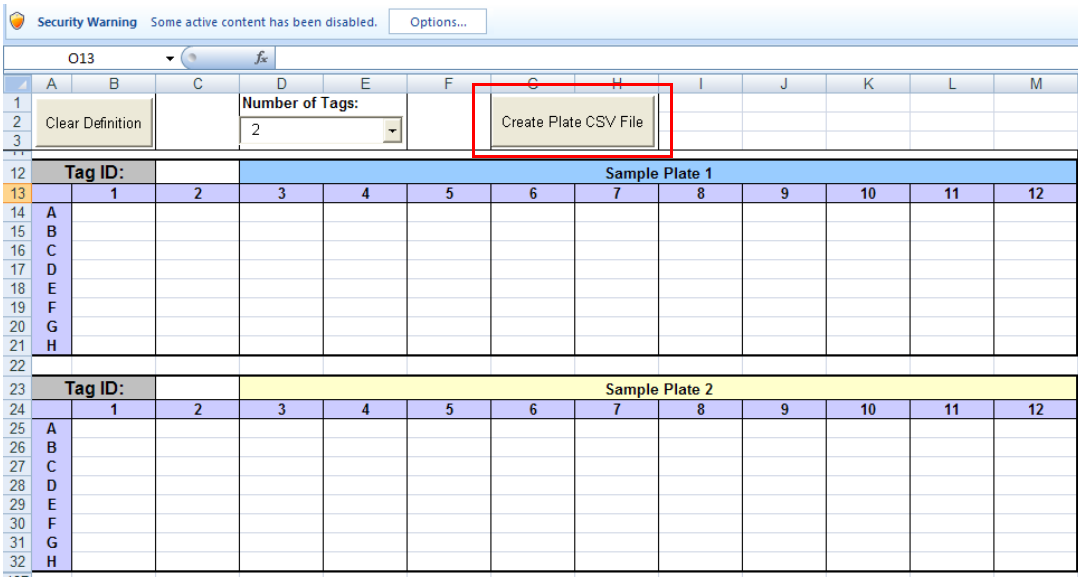
4. Select **Enable this content** from the Microsoft® Office Security Options dialog box.



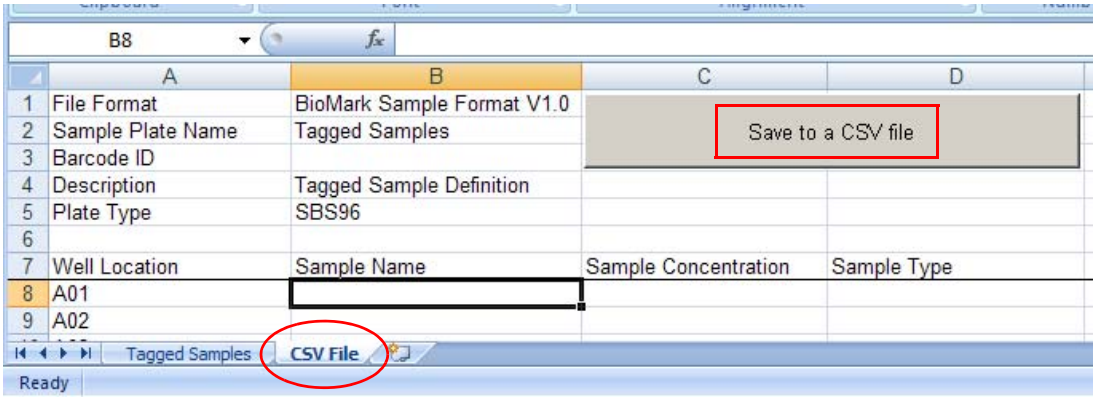
5 Click **OK**.

6 Edit the Microsoft® Excel™ template to match your experiment

7 Click **Create Plate CSV File**.



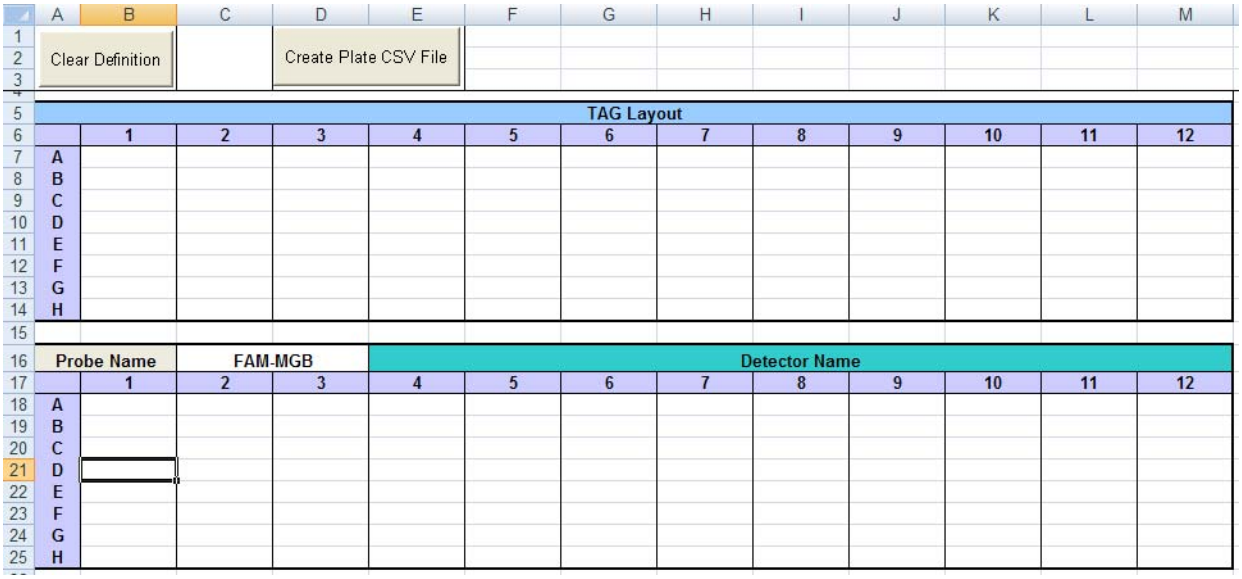
8 Open the new CSV file tab and double-check your annotations.



9 Click **Save to a CSV file** to save the file and to select a convenient location for future retrieval.

Assay Setup

1 Next, open the **AssayPlateDefinitionForMoreS** file.



2 Edit the Microsoft Excel file to match your experiment.

3 Click **Create Plate CSV File**. A new tab, CSV file, is added to the Excel file.

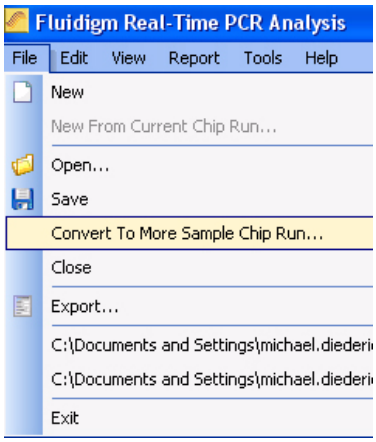
4 Open the new CSV file tab and double-check your annotations.

5 Click **Save to a CSV file** to save the file and to select a convenient location for future retrieval.

Import the Templates

Import the Sample template files.

1. Open the SNP Genotyping Analysis software.
2. Open a chip run that you wish to annotate.
3. Select **Sample Setup** in the Chip Explorer.
4. Click **Import** in the Task pane.
5. Browse to the location where you saved your sample template.
6. Click **Open**.
7. Go to **File > Convert to More Samples Chip Run.**



Import the Assay template files.

1. Open the SNP Genotyping Analysis software.
2. Open a chip run that you wish to annotate.
3. Select **Assay Setup** in the Chip Explorer.
4. Click **Import** in the Task pane.
5. Browse to the location where you saved your assay template.
6. Click **Open**.
7. Go to **File > Convert to More Samples Chip Run.**

Viewing Chip Run Data in the Data Analysis Software

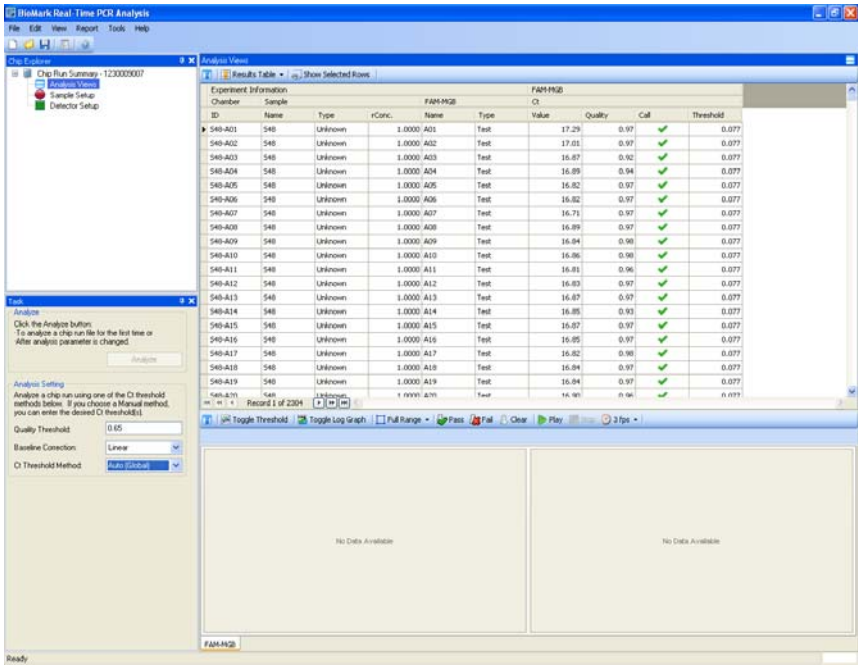
3

In this chapter:

Analysis Settings

Change settings in the analysis settings pane.

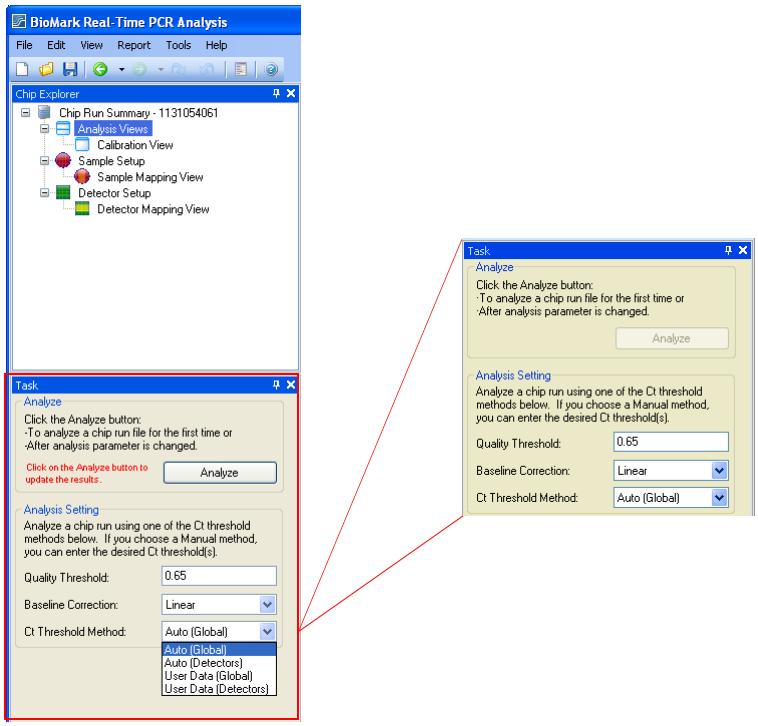
1. Launch the BioMark Real-Time PCR Analysis software.
2. Click **Analysis Views** in the chip explorer pane.



Changing C_t Threshold Methods

Within the analysis settings pane, in the Task frame, choose from four different C_t threshold methods to view your data.

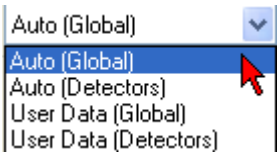
- Auto Global
- Auto Detectors
- User Data Global
- User Data Detectors



Each option is described in the following sections.

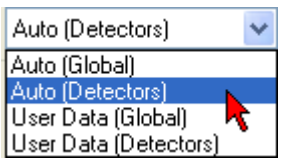
Auto Global

This option automatically calculates a threshold that is applied to the entire chip.



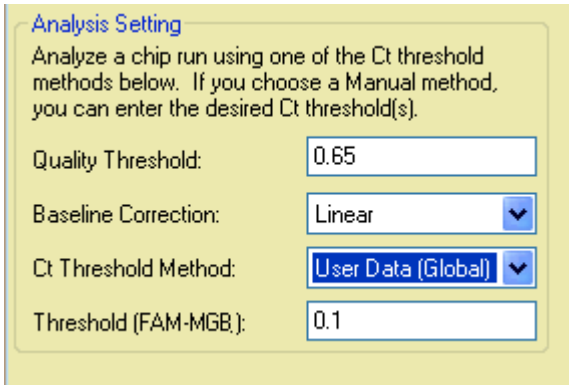
Auto Detectors

This option independently calculates a threshold for each detector on a chip. If you use this option, you must enter a unique detector name in the Detector Editor during detector set up.



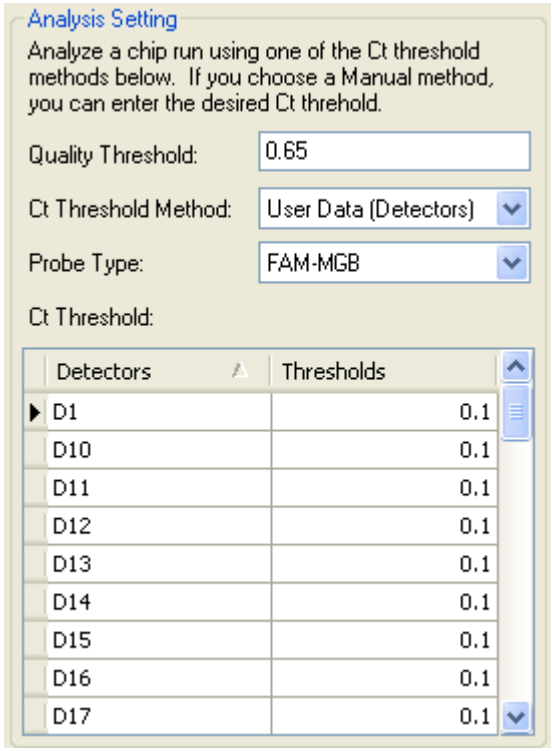
User Data Global

This option allows you to manually adjust the threshold when searching for the C_t rise in slope. The value you supply is applied to all the detectors.



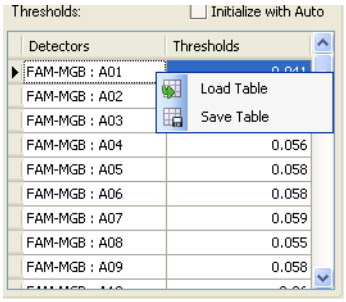
User Data Detectors

If you are using multiple detectors on a chip and you want tighter control when searching for the C_t curve's rise in the slope. You can individually set the threshold for each detector.



Loading and Saving User-Defined Threshold Settings

For copying user-defined threshold setting from one chip run to another, right-click the threshold table and choose load or save.

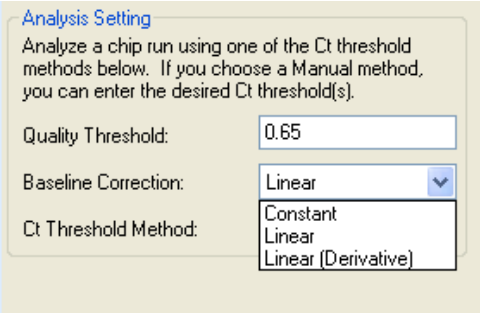


Right-click to open

Baseline Correction

The baseline correction drop-down menu has three options:

- Constant
- Linear (default)
- Linear (Derivative)

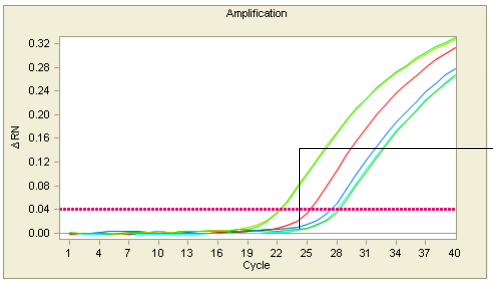


Using Linear Baseline Correction

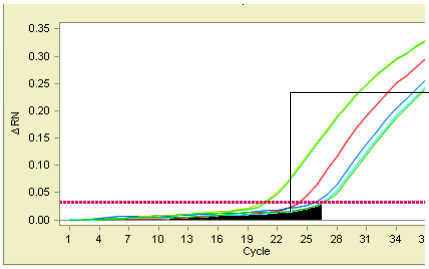
Use the Linear baseline correction when the amplification is low, producing higher C_t values. Linear baseline correction eliminates baseline 'drift' by flattening the baseline.

Using Linear (Derivative) Baseline Correction

An additional method of baseline correction with a more robust handling of nonlinear baselines and their impact on C_t estimates.



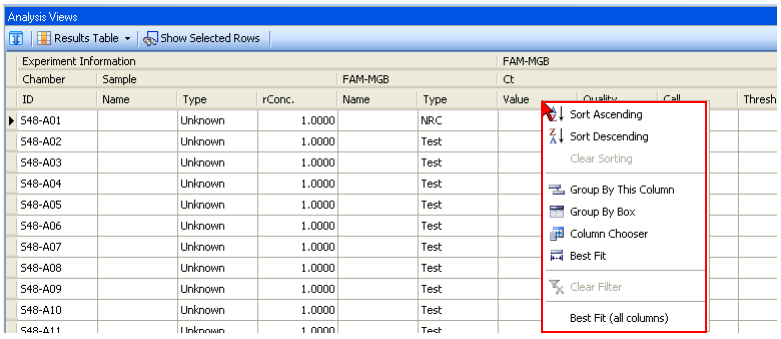
Notice how the baselines are flattened in the Linear correction, above, compared to the rising baselines in the Constant correction shown below as black fill.



Using the Column Header Right-Click Menu

In the Results Table view, right-click a column header to:

- Adjust columns, see page 64
- Group columns, see page 65
- Sort columns, see page 71
- Column Chooser, see page 72
- Customize search filters, see page 76

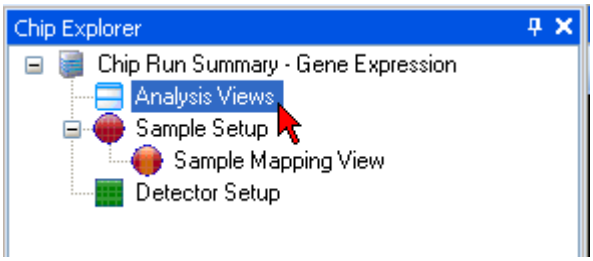


In this example, right-click the Value column header to open the options menu.

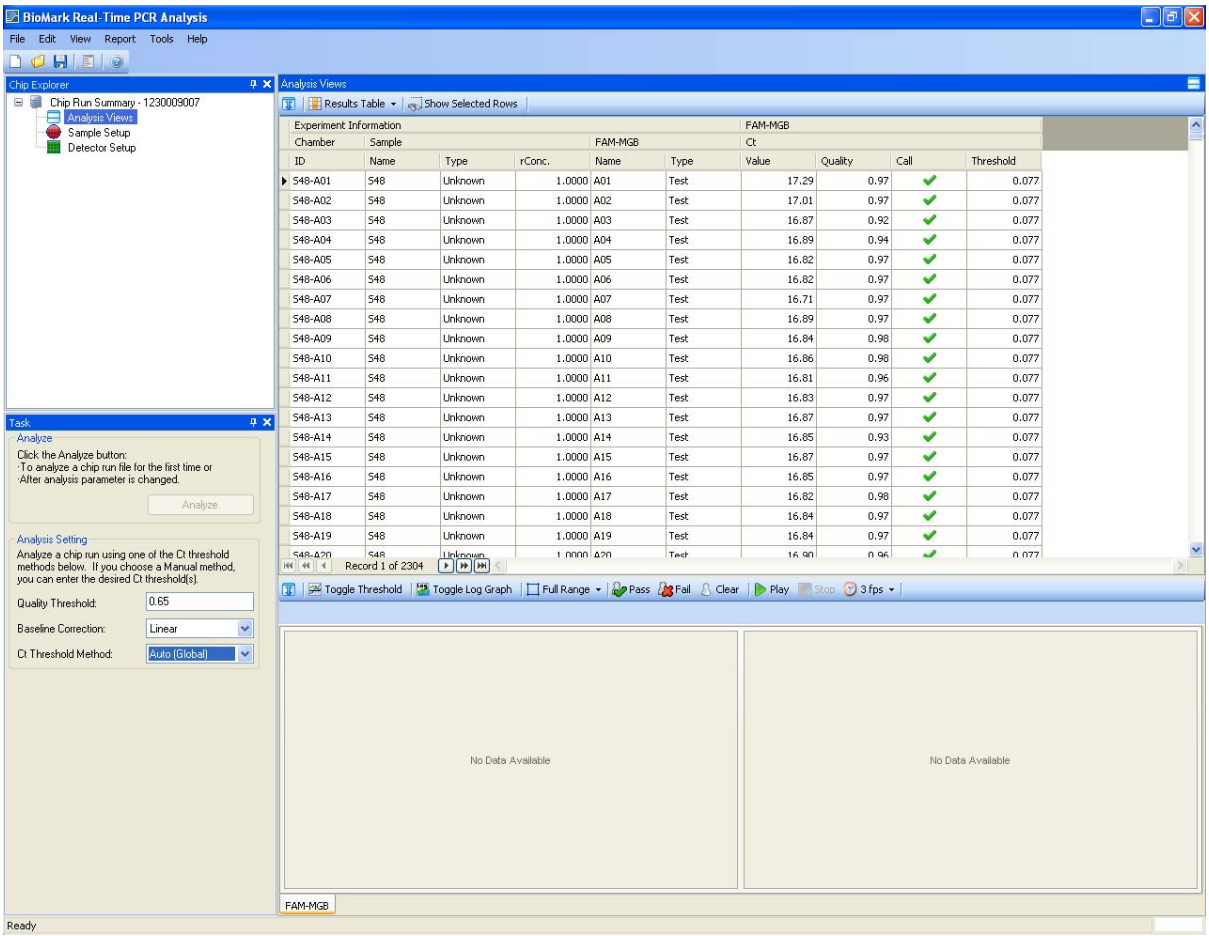
Using the Results Table

Accessing the Results Table

1 Click **Analysis View** in the BioMark Real-Time PCR Analysis software.



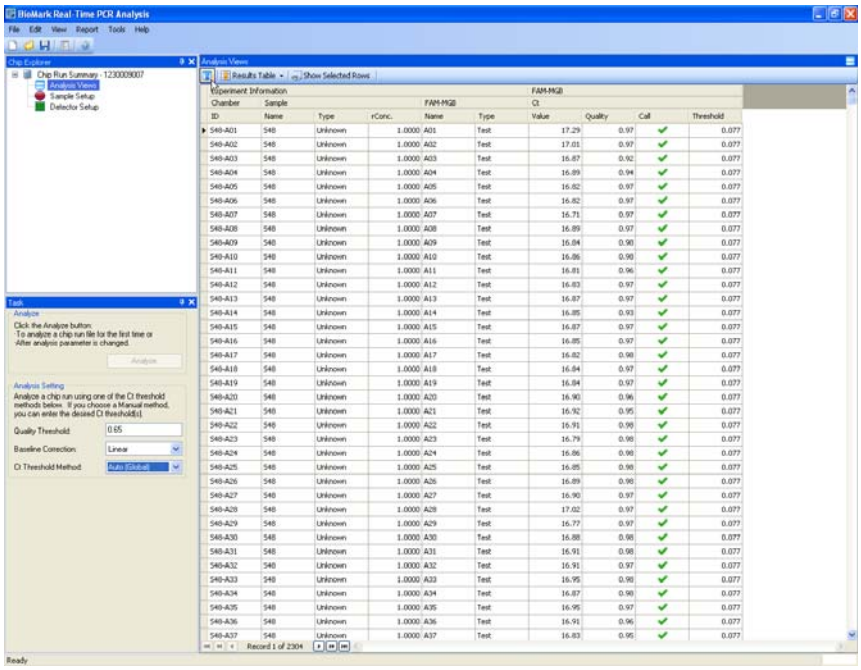
The Results Table is the default window.



Expanding to Full View

Click the double-arrow button to expand the view.

NOTE: This also works for the Image View and the Results Table.

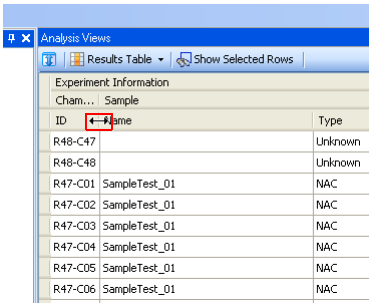


Adjusting Columns Using the Cursor

- Position your cursor on a separator line and when it changes to a double arrow, drag the column bigger or smaller.

Or,

- Double-click on the separator line to adjust to precisely fit the contents of columns (same as 'Best Fit' described below).

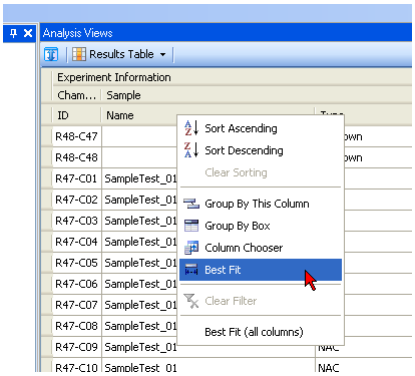


Adjusting Columns Using ‘Best Fit’

- Right-click a column header (*Name* in this example) and then click **Best Fit**. The column automatically adjusts to precisely fit the contents of the selected column.

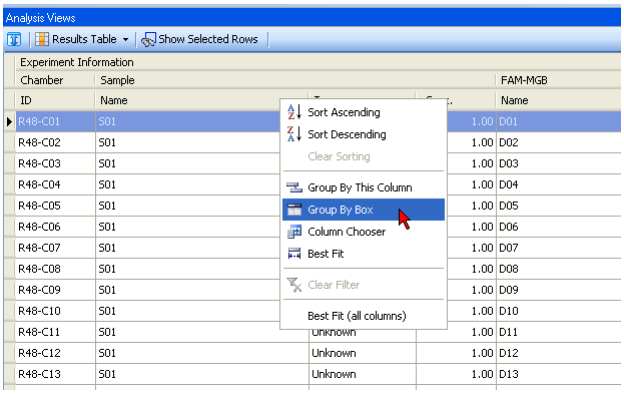
Or,

- Click **Best Fit (all columns)** to adjust all columns simultaneously.

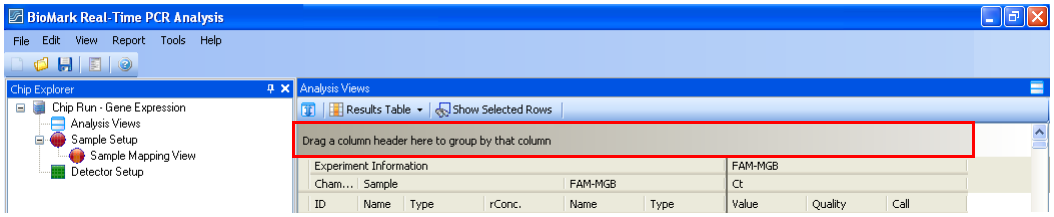


Grouping Two or More Columns

1. Right-click on any column header.
2. Click **Group by Box**.

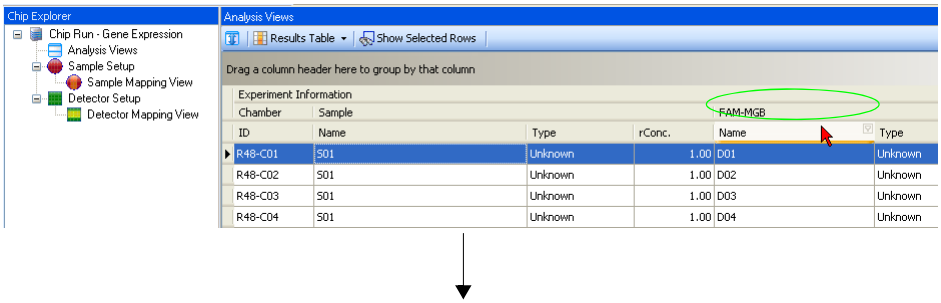


The Grouping bar appears.

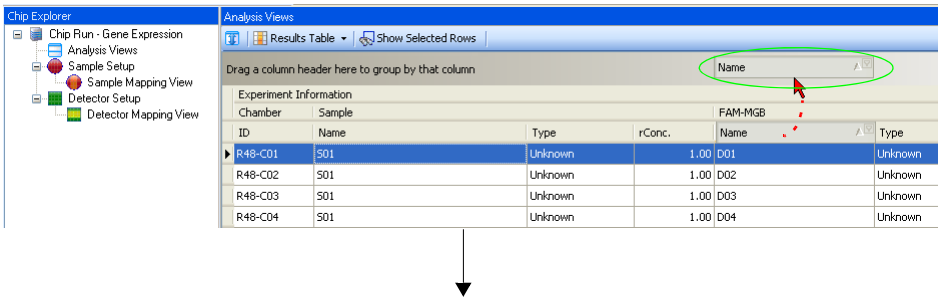


3 Click the column header that you want to group and, while holding down the mouse button, drag it to the bar as shown below.

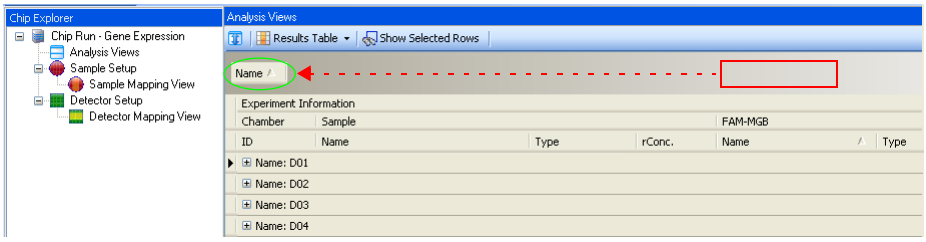
a. Click and hold mouse button on header



b. Drag to any place on the bar

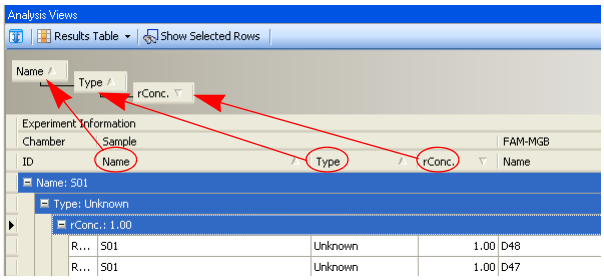


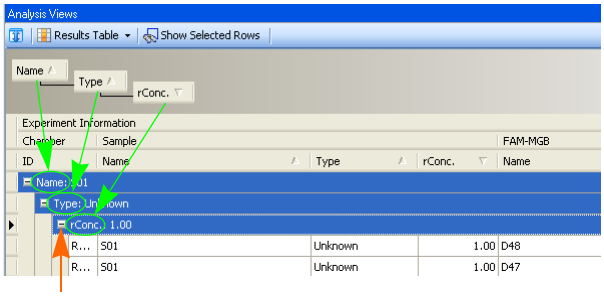
c. Release mouse button and header snaps to position at the left



The data are now grouped by name in the Results Table.

4 Group as many elements as you like by dragging and dropping, as in the example below.

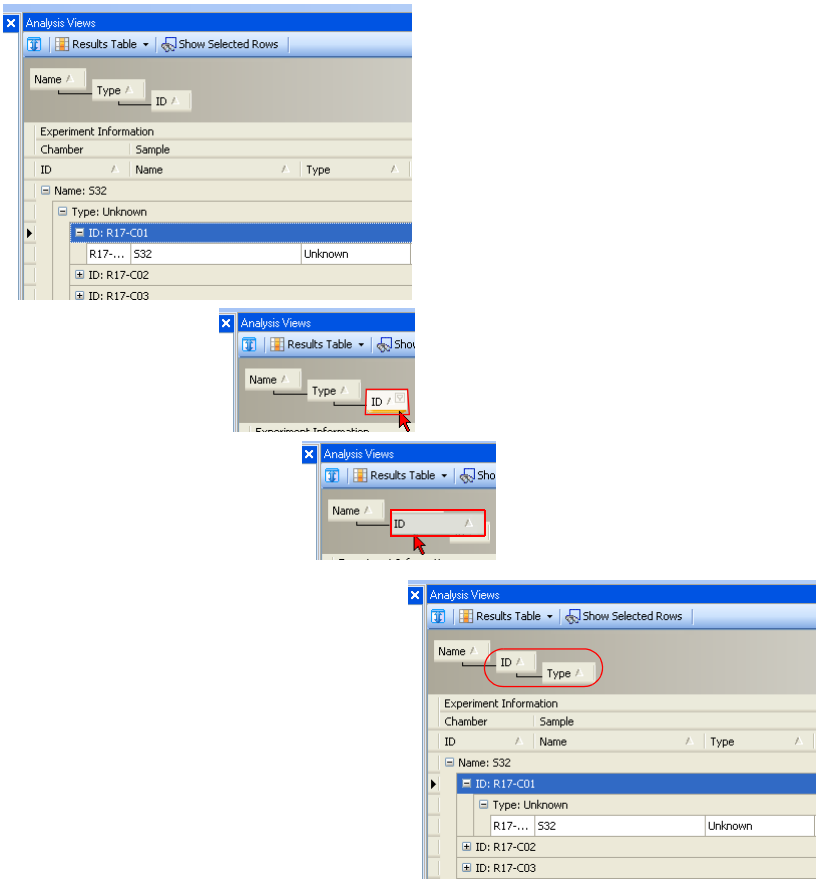




Click + (plus) or – (minus) to expand/collapse the windows.

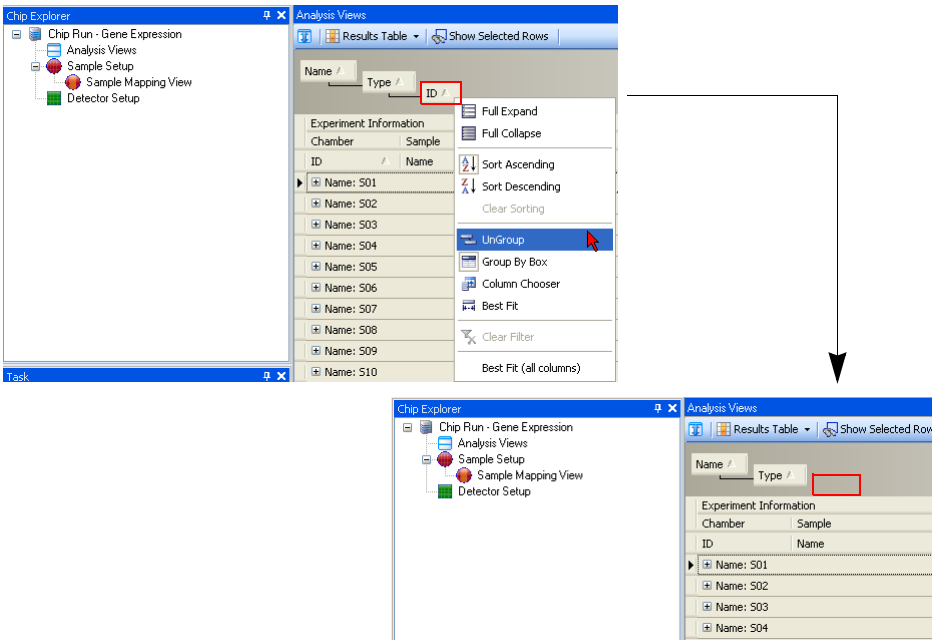
5 Drag and drop one header element over another as shown below to change places (hierarchy). The hierarchy dictates how the data displays as you expand windows.

In this example, the *ID* header is dragged over the *Type* header and then dropped. They exchange places as a result.



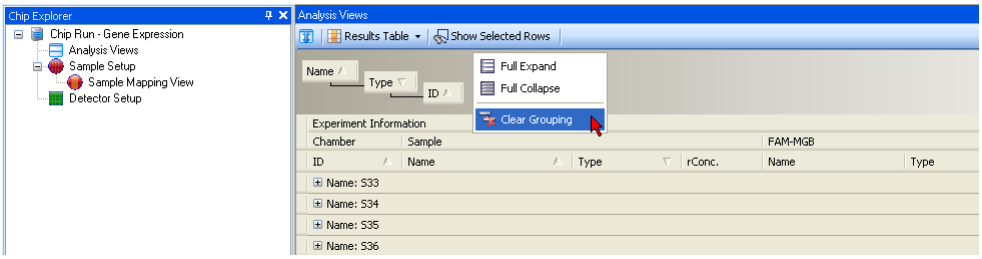
Ungrouping One Header

1. Right-click a header within a group.
2. Click **Ungroup** to remove the header from the group.



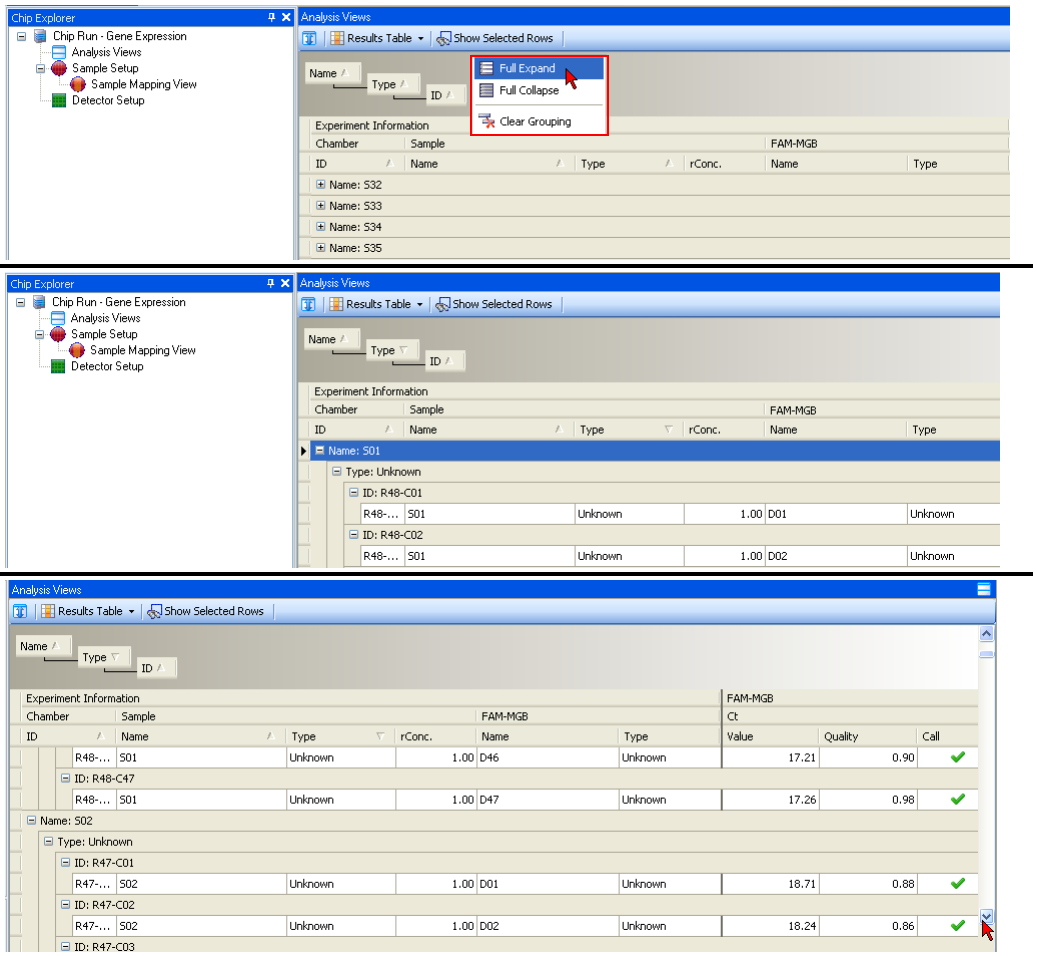
Ungroup All Headers

1. Right-click anywhere on the grouping bar.
2. Click **Clear Grouping**.



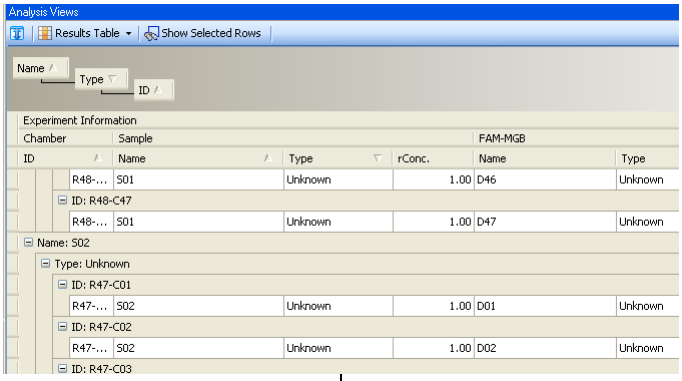
Expanding and Collapsing All

1. Right-click anywhere on the grouping bar.
2. Click **Full Expand**. The grouped windows expand as shown below.

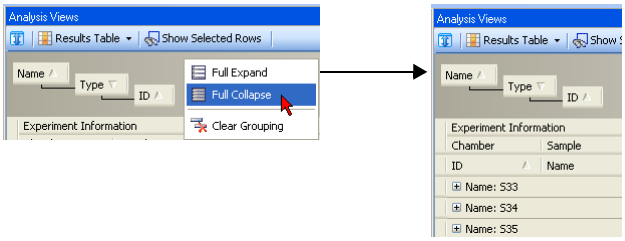


3. Collapse all by right-clicking anywhere on the grouping bar.

4 Select **Full Collapse**.



Before full collapse

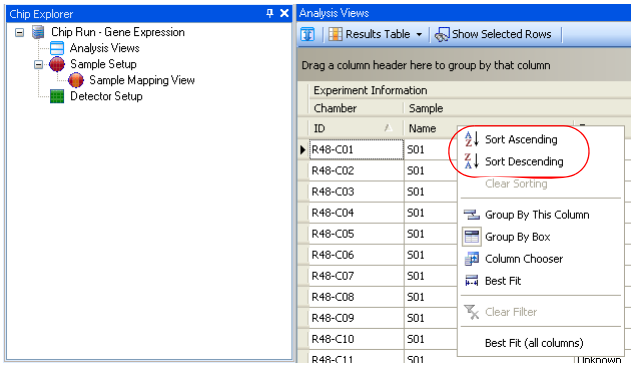


After full collapse

Sorting

1 Right-click a column header.

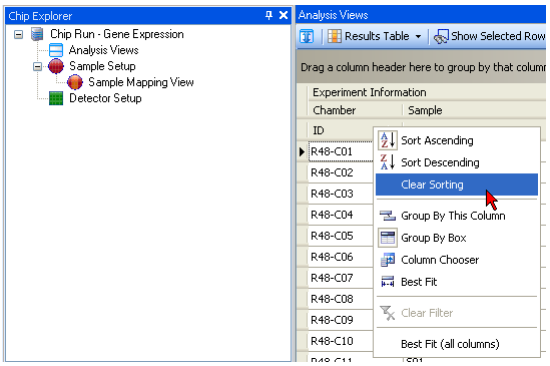
2 Choose either **Ascending** or **Descending** to sort that column accordingly.



Unsorting

1 Right-click a sorted column header.

2 Click **Clear Sorting**.

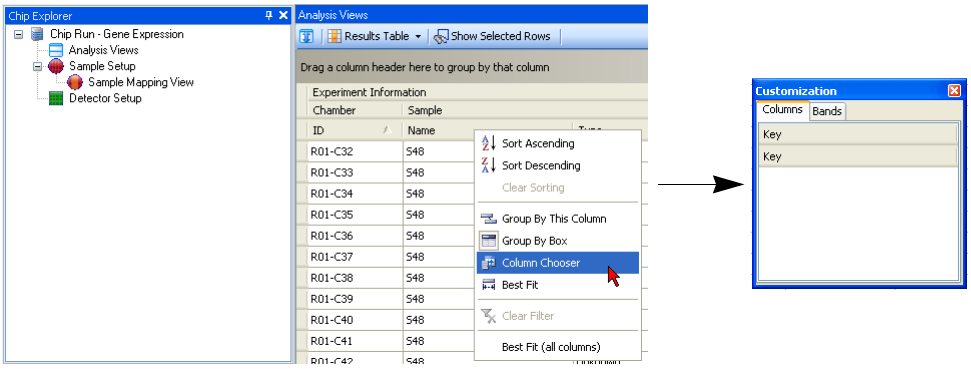


Column Chooser

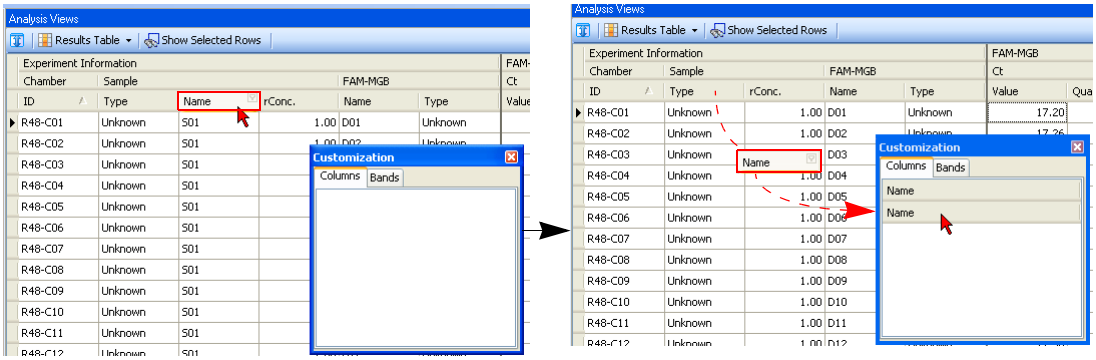
Depending on how you set up your sample plate and detector plate, you can have 20+ columns in the Results Table pane, all of which are not viewable at once. To temporarily remove columns not of immediate interest, follow the procedure below.

1 Right-click a header.

2 Click **Column Chooser**. The Customization dialog box opens.



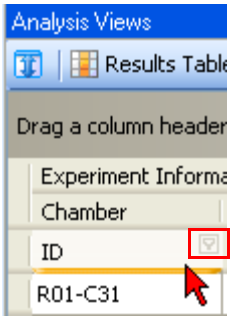
3 Drag and drop unwanted column headers onto the Customization dialog box.



4 Replace the column headers by dragging them from the Customization dialog to their original position.

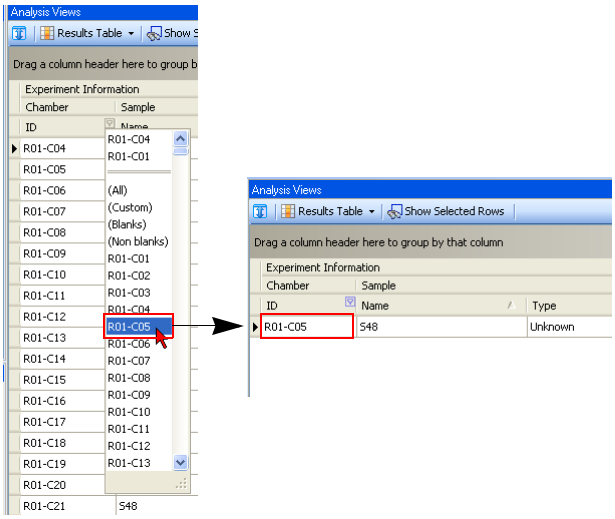
Drop-down Menus on the Column Headers

Each column header has a drop-down menu. Place your cursor over a header to reveal the drop-down menu symbol.

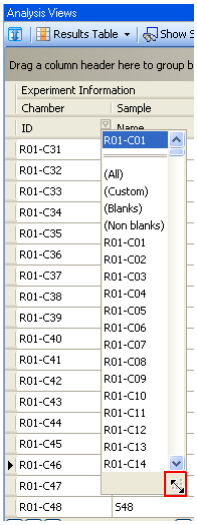


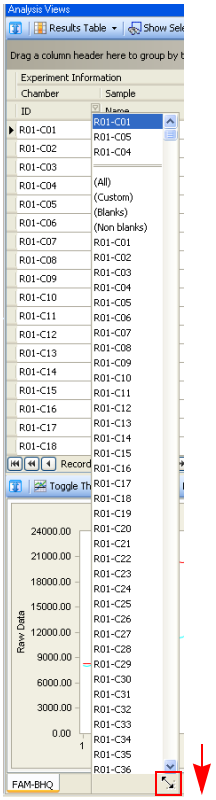
1 Click the drop-down menu symbol to display the menu.

2 Click a location to go to that location.



3 Click and drag the drop-down menu to size it.



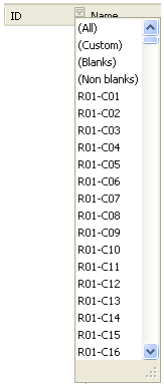


Header-specific Menu Options

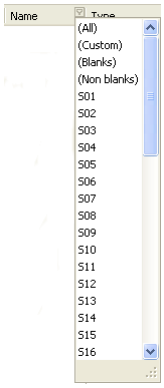
Options on the column header drop-down menus are described in the following sections. Each column header menu has options specific to that header.

The following figure shows an example of each header-specific drop-down menu.

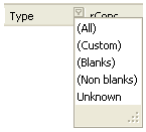
ID



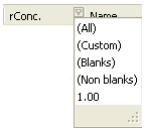
Sample Name



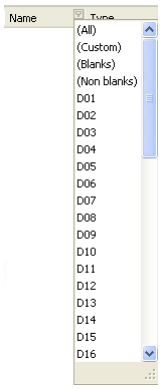
Type



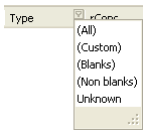
rConc.



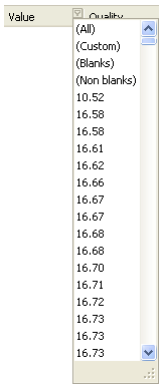
Detector Name



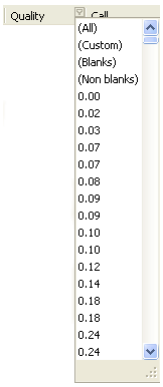
Type



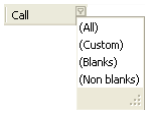
Value



Quality



Call

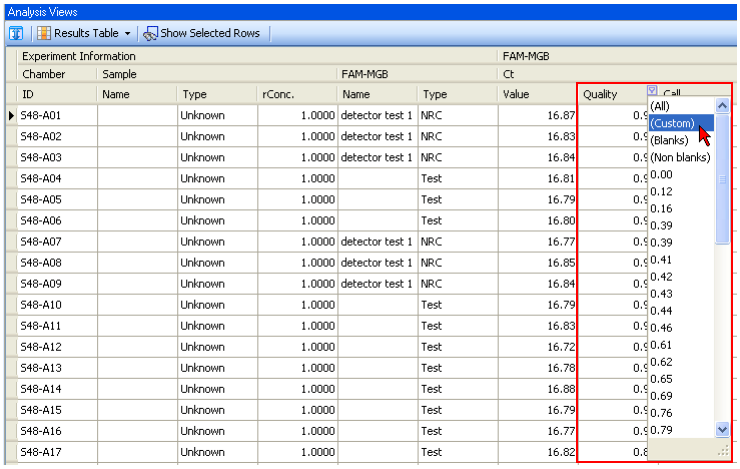


Custom Filters

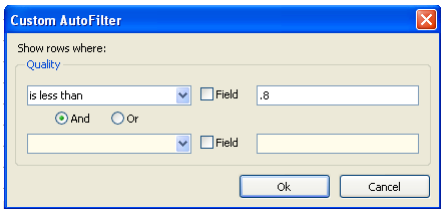
Use filters to narrow your search for a particular parameter. In the following example, we isolate C_t quality values below 0.8.

1 Click the Quality header drop-down menu.

2 Click **Custom**.



The Custom AutoFilter dialog box opens.



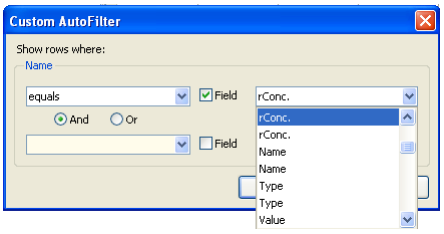
3 Delimit your search:

a Select a filter from the first drop-down menu.

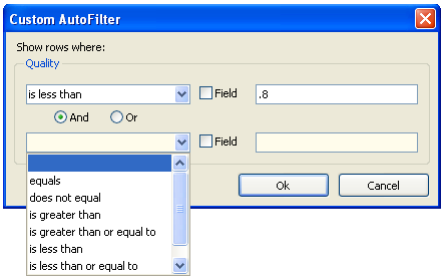
b Enter the target value (0.8 in this example) in the Field text box.

Or,

c Click the Field check box to activate the drop-down menu, and then select a filter.



4 (Optional) Continue delimiting your search by clicking **And/Or** and then selecting filters from the drop-down menus.



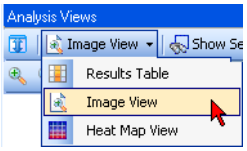
5 Click **OK**.

Using the Image View

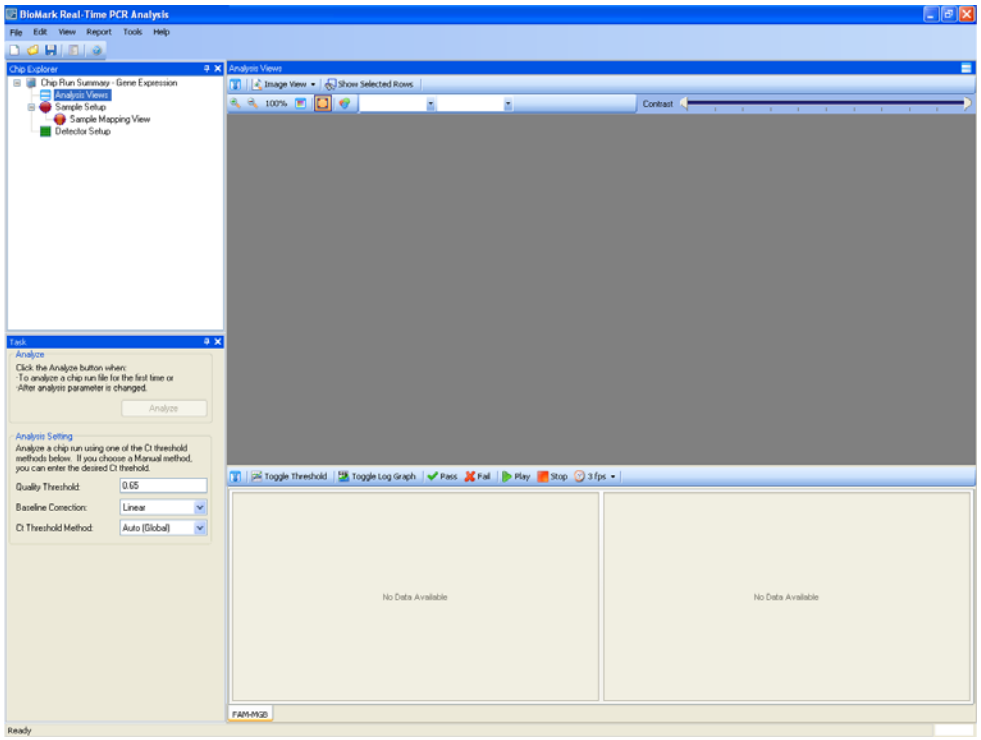
View images from individual cycles in this window.

1 Click the Results Table drop-down menu.

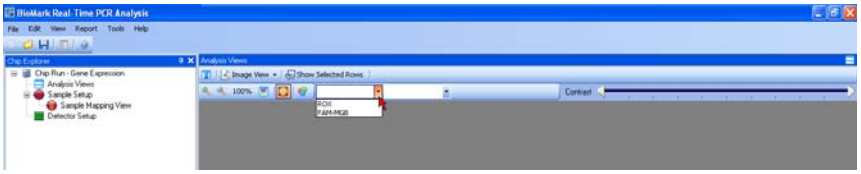
2 Click **Image View**.



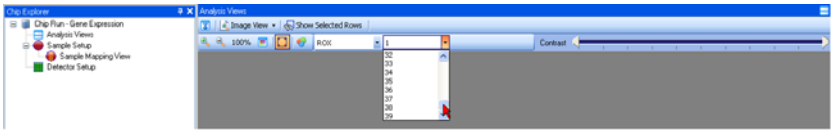
The default Image View window opens.



3 Select a dye from the drop-down menu.

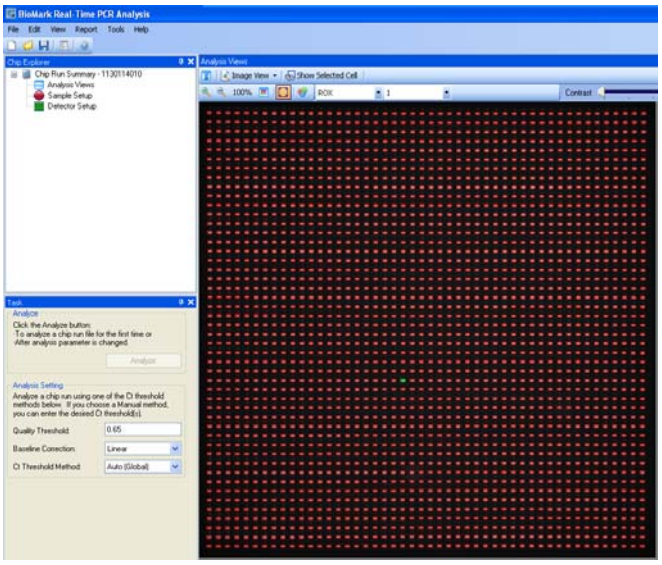


4 Select a cycle number of interest from the Cycle Selection drop-down menu.



IMPORTANT: An image displays only after you have selected a dye and a cycle number.

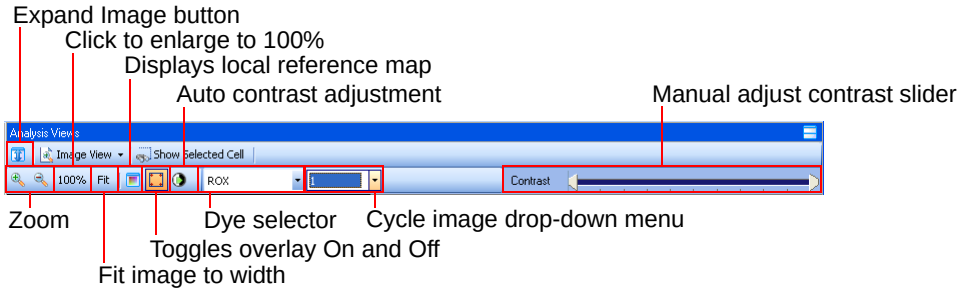
A representation of the chip displays in the Image View.



5 (Optional) Click the double arrow to expand the image.

Image View Tool Bar

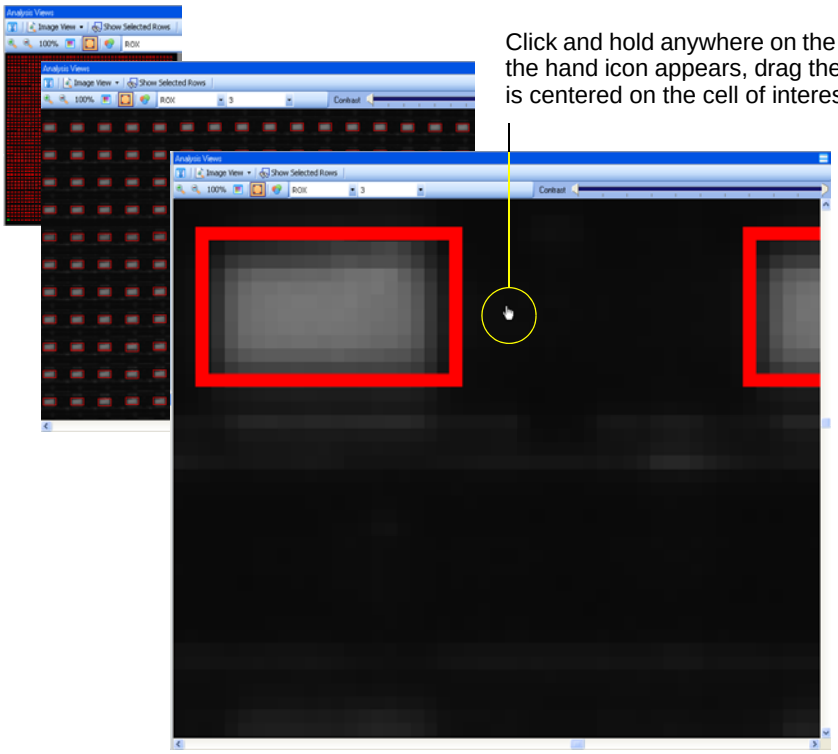
Elements of the Image View tool bar are shown below.



Zoom

You can increase or decrease the image view size in several ways:

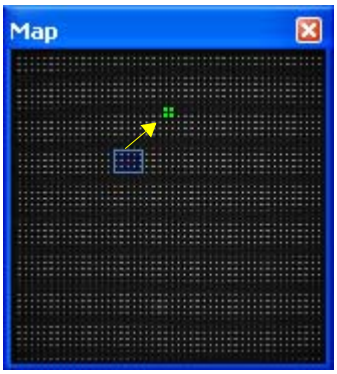
- Multi-clicking the magnifying glass icons (+ and –).
- Click the 100% icon.
- Clicking the Fit icon to fit image to width.
- Clicking inside Image View and then rolling the mouse scroll wheel (up/backward = larger, down/forward = smaller).



Location Reference Map

Use the location map to reference your cell of interest within the entire framework of the chip.

1. Click the Location Reference map icon to open the map.
2. Click and drag the blue rectangle to a location of interest. In the example below, the blue rectangle within the Location Reference map is dragged to the green cells which enlarges the green cells in the Image Viewer.



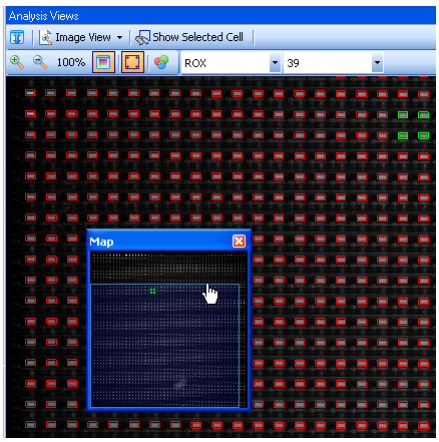
Drag blue rectangle to area of interest

Dragging the blue rectangle to an area of interest enlarges that area in the Image Viewer as shown here.

Adjusting the Size of the Location Reference Map

The size of the image in the Image Viewer determines the size of the blue rectangle in the Location Reference Map.

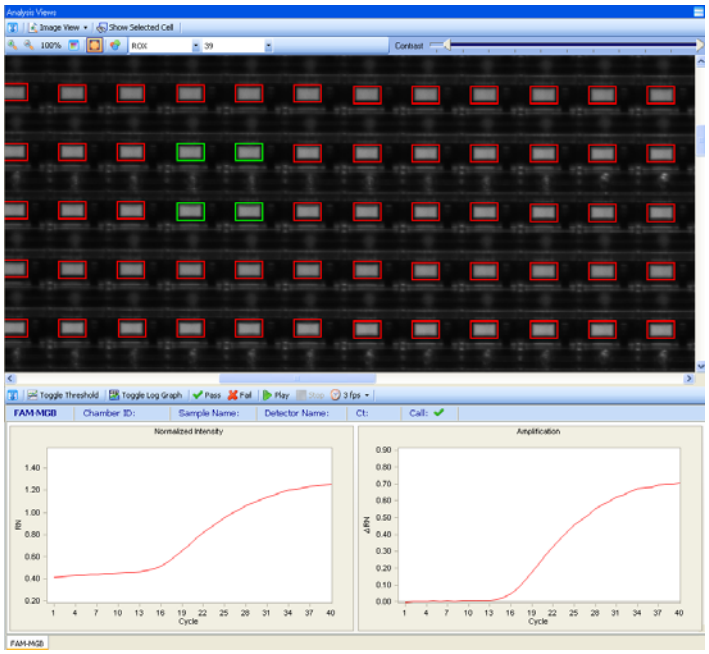
In the example below left, the image is has not been zoomed so the blue rectangle on the map is large. In the example below right, the image has been enlarged (by clicking in the Image View and then rolling the scroll wheel on the mouse).



Here, the blue rectangle is large and cannot be dragged with much accuracy.

Here, the image has been enlarged so that the blue rectangle is smaller and easily dragged to an area of interest.

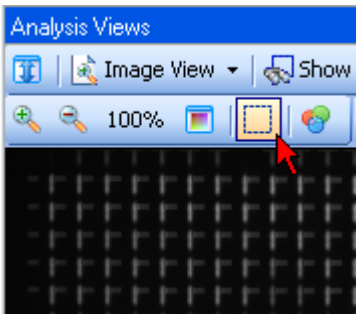
NOTE: Selected cells in the Image View are also displayed in the Graph View as shown below.



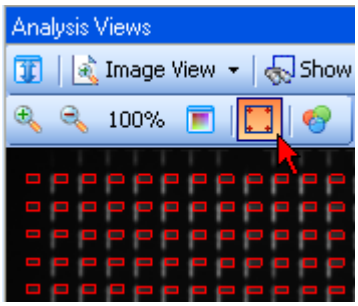
Raw data, amplification plots, and dye intensities are displayed in the Graph View when cells are selected in the Image View.

Overlay

1. Click the Overlay icon to activate the red-square grid.
2. Click the Overlay icon again to inactivate the red-square grid.



Toggle grid *off*



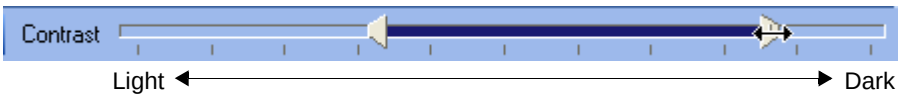
Toggle grid *on*

Contrast

Adjust image contrast:

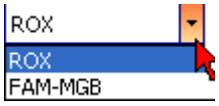
- Click the Auto-Contrast icon.

  Or,
- Move the contrast sliders by placing your cursor over a slider, then click and drag.



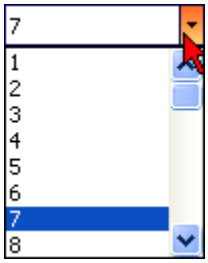
Dyes

Change the dyes in the image viewer from the drop-down menu.



View Image in Each Cycle

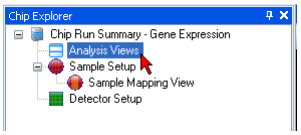
Use the drop-down menu to select an image to view. Select number 7 in the menu, for example, and the image taken at cycle 7 displays in the Image Viewer.



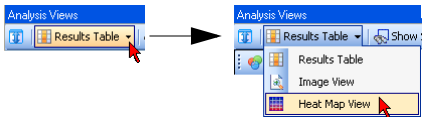
Using the Heat Map

The heat map color codes C_t values for easy reference.

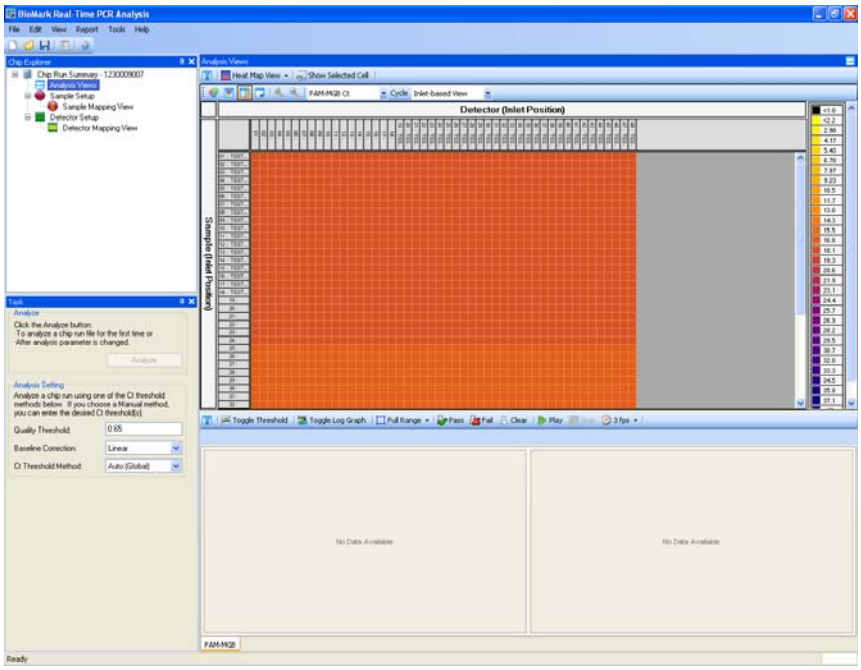
1 Click **Analysis View** in the BioMark Real-Time PCR Analysis software.



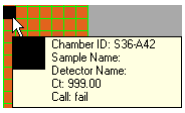
2 Click **Heat Map View** from the Results Table drop-down menu.



The default heat map opens.



NOTE: A black square indicates a failed call as shown in the example below.

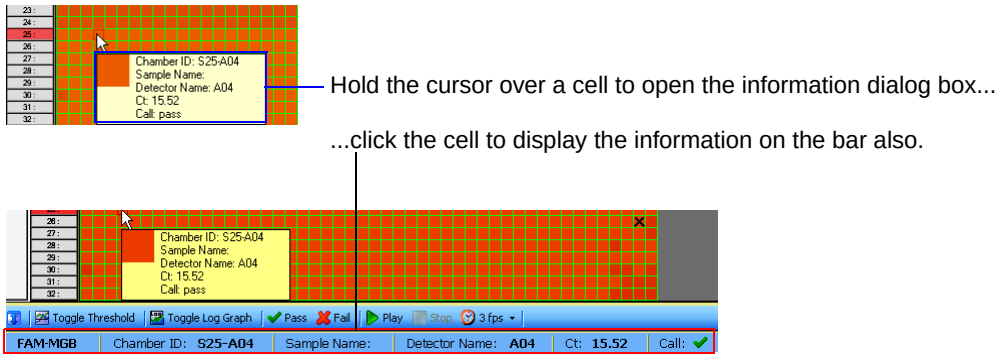


Also, negative controls that do not show amplification appear as black squares.Option

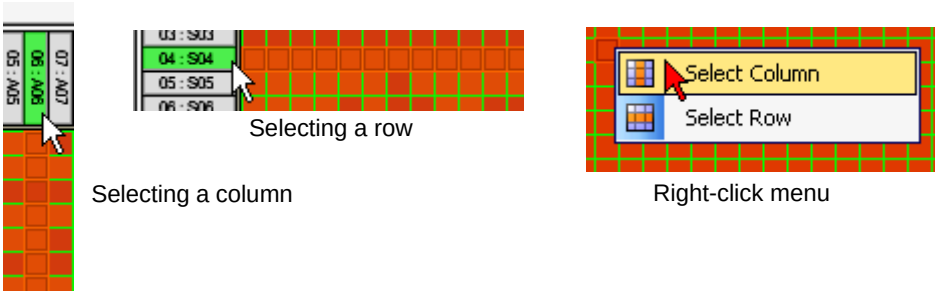
NOTE: An **X** signifies a questionable amplification curve.

3 (Optional) Click the double arrow to expand the image.

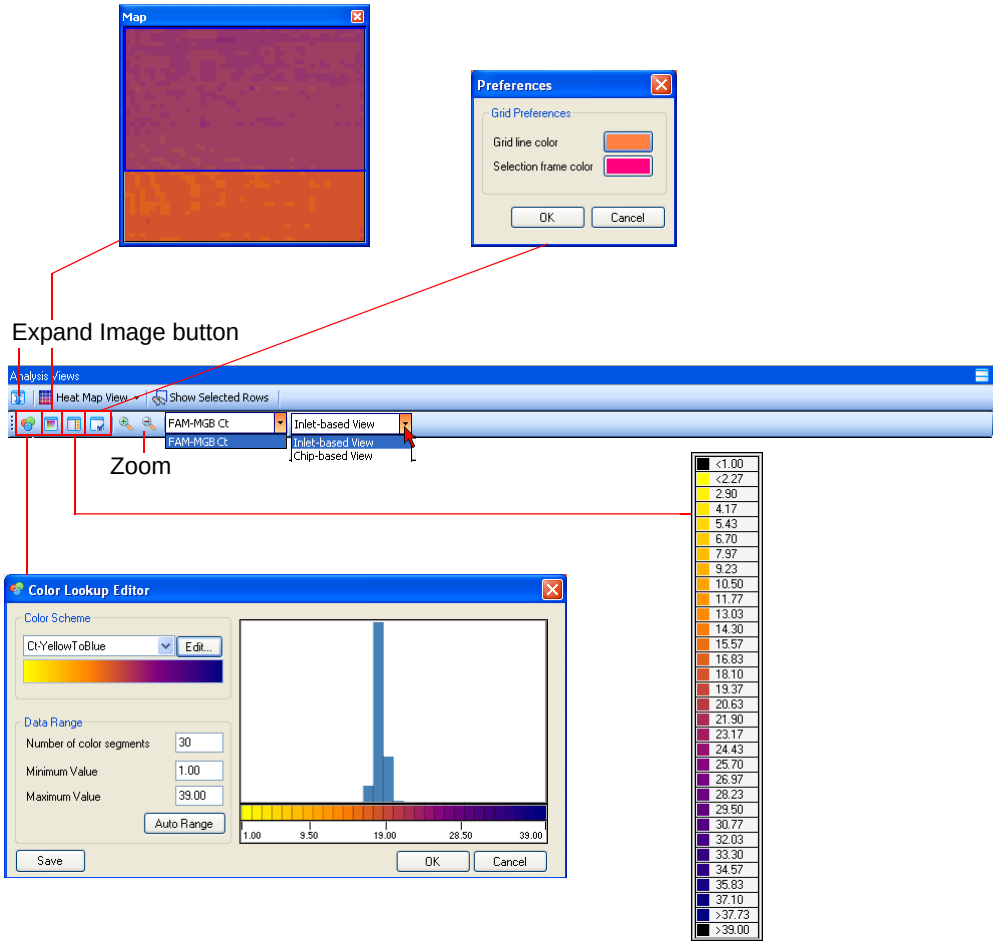
4 (Optional) Hold your cursor over a cell of interest and an information dialog box opens; click the cell and the information appears on the task bar.



5 (Optional) select a row or a column by clicking an inlet or using the right-click menu as shown below.



Heat Map View Tool Bar

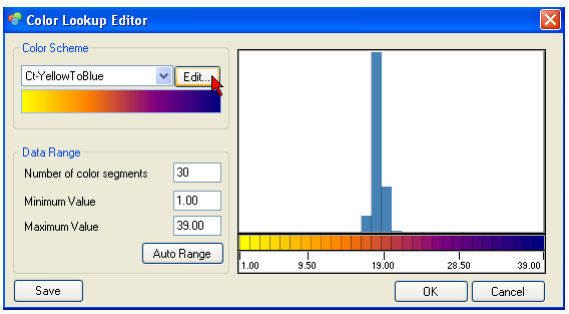


Color Lookup Editor

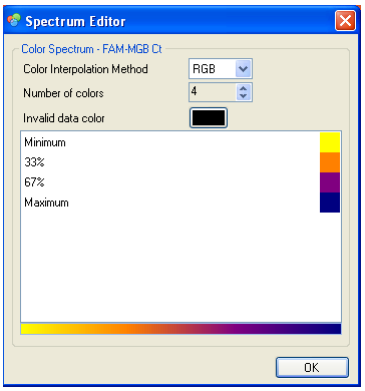
Define a range of valid C_t values using the color editor.

1 Click the Color Lookup Editor button.

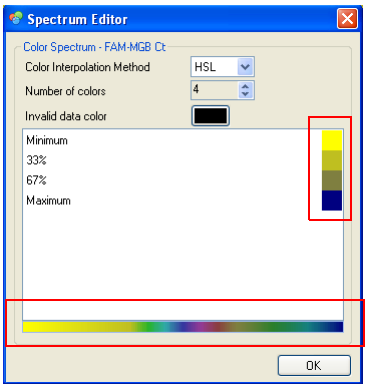
2 Click **Edit**.



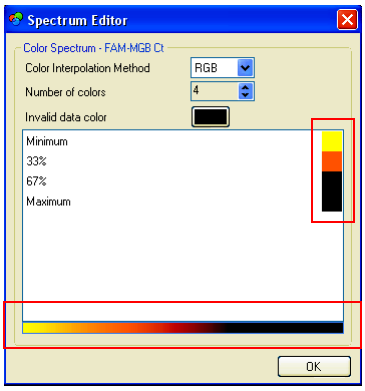
The Spectrum Editor opens.



3 Choose **RGB** (red, green, blue) or **HSL** (hue, saturation, lightness) from the Color Interpolation Method drop-down menu.



HSL



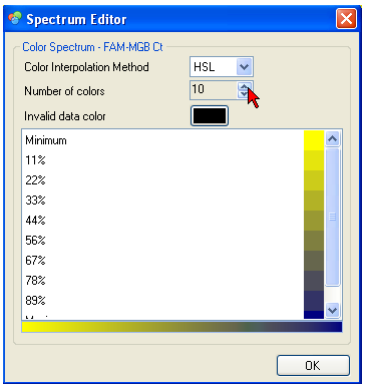
RGB

4 (Optional) Change the percentage increments between colors by changing the number in the Number of Colors spin box.

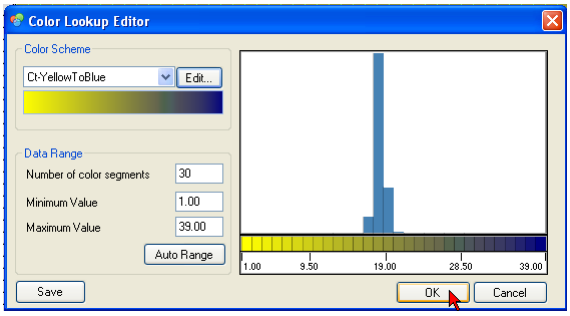
a Click **Edit**.

b Change the value in the Number of Colors spin box (from 1 to 20).

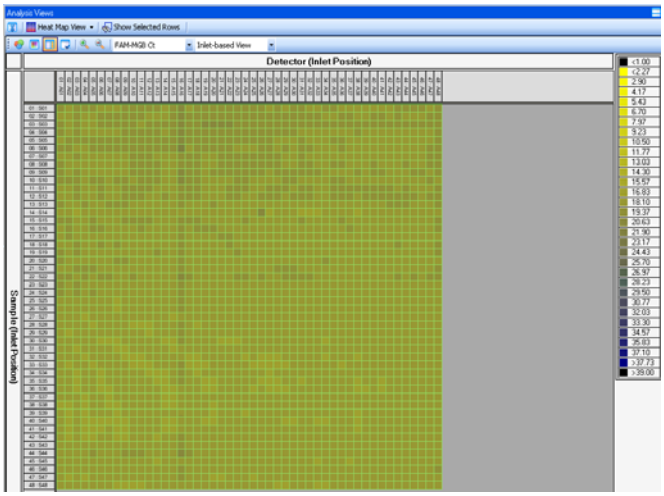
c Click **OK**.

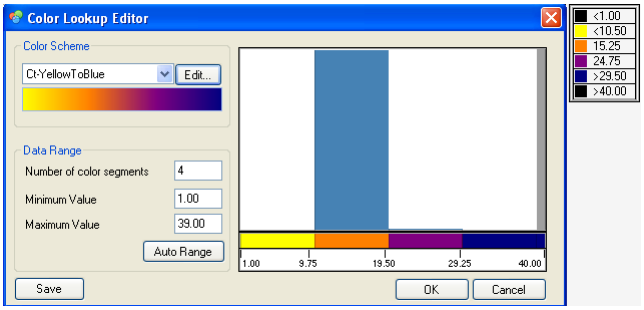


d Click **OK** again.

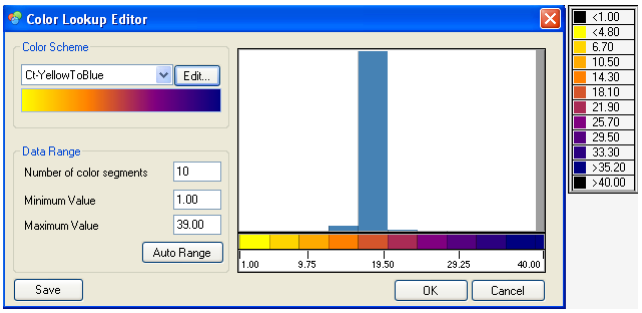


The change (10 colors) is reflected in the heat map and in the legend.

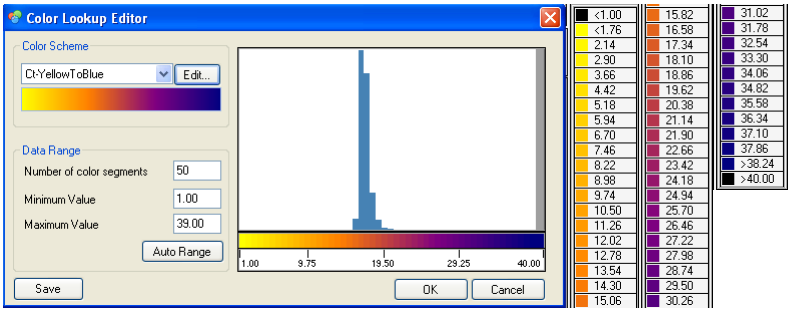




Using 4 colors



Using 10 colors



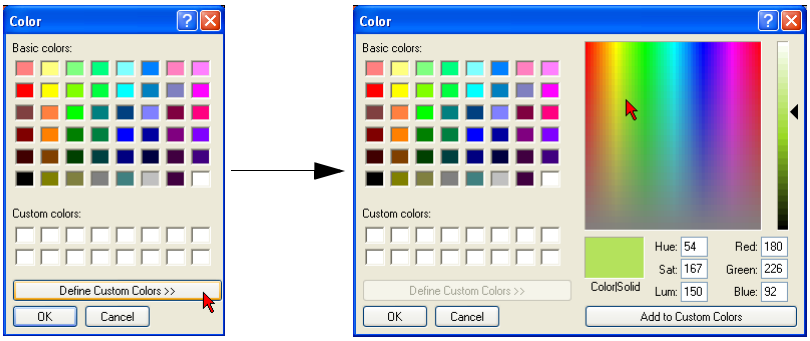
Using 50 colors

5 (Optional) Click **Invalid Color Data**  to change the color.

a Click a color square.

Or,

b Click **Define Custom Color** to pick a color other than a basic color.

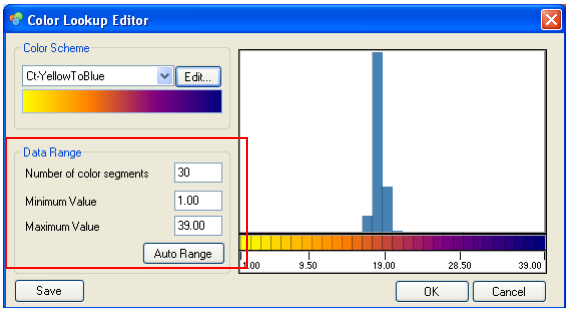


c Click **OK**.

Color Range Pane in the Color Lookup Editor

You can change the following parameters:

- Number of color segments
- Minimum value
- Maximum value
- Auto Range



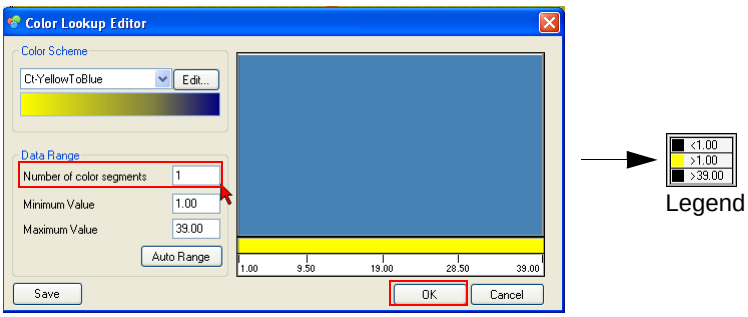
Changing the Number of Color Segments

Change the segments shown in the heat map from 1 to 2034.

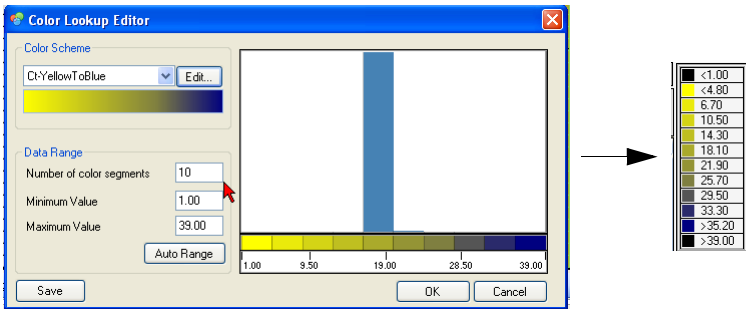
1. Type a value in the Number of Color Segments text box.
2. Click **OK** to reflect changes in the legend.

   The examples below illustrate that the greater the number of color segments, the finer distinction between legend values.

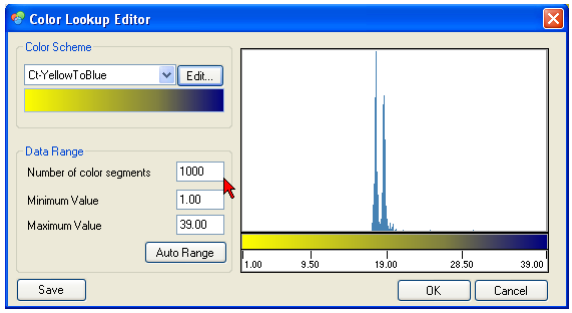
1 Color Segment

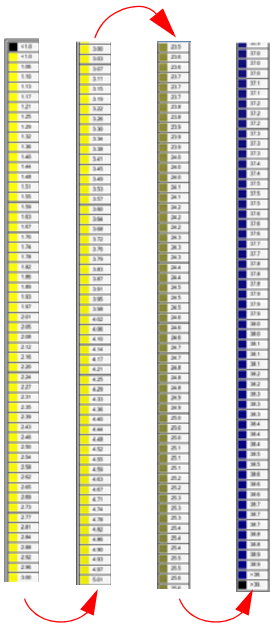


10 Color Segments



1000 Color Segments



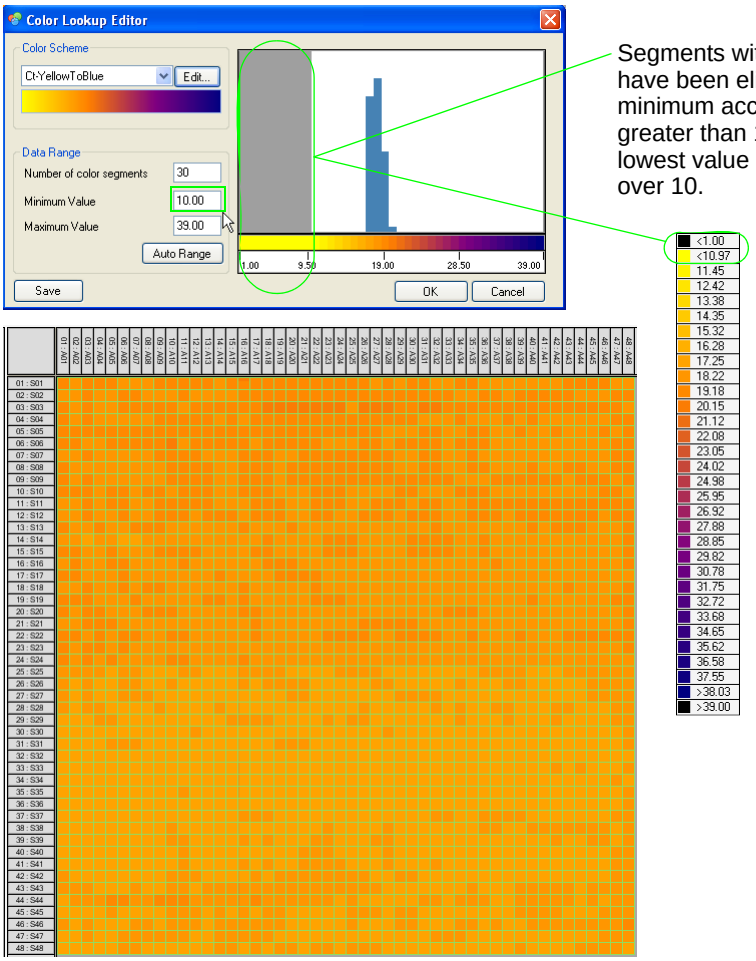


Changing Minimum Values

Change the minimum value when you want to exclude a segment from the lower range. For example, changing the value from 1 to 10, excludes any C_t value from 1 to 10 as the example below illustrates.

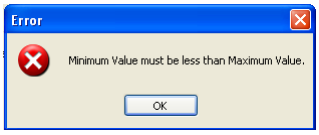
1 Enter a value in the Minimum Value text box.

2 Click **OK**.



Segments with values from 1 to 10 have been eliminated as the minimum acceptable value is now greater than 10. As a result, the lowest value on the legend is now over 10.

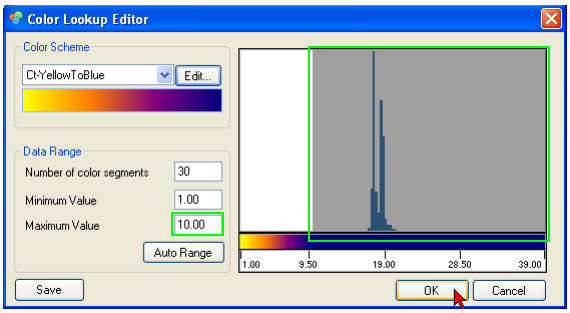
NOTE: If you attempt to input a minimum number greater than the maximum value, this error appears:



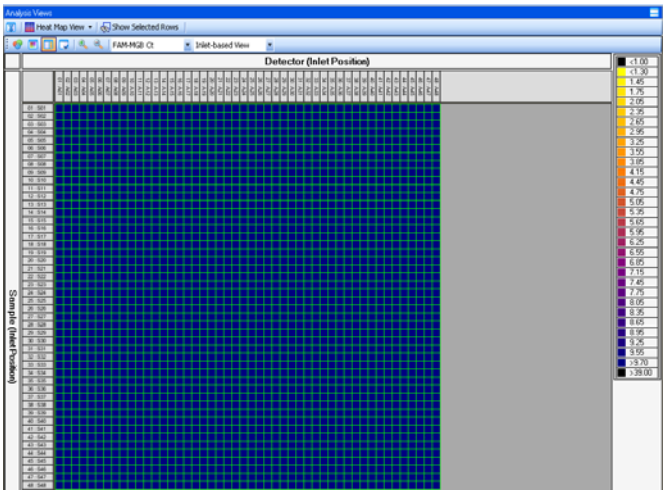
Changing Maximum Values

Change the maximum value when you want to exclude a segment from the higher range (from 1 to 39). For example, changing the maximum value from 39 (default value) to 10, any C_t values above 10 are excluded (gray area), as the example below illustrates.

1. Enter a value in the Maximum Value text box.
2. Click **OK**.



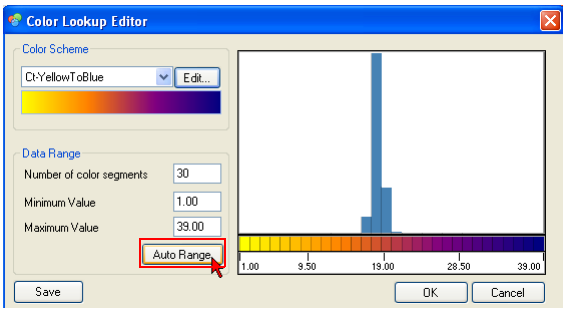
When you click **OK**, the heat map and the legend reflect the change also. Since the C_t values of the cells of interest are above 10, they have been eliminated. As a result, the heat map is almost exclusively dark blue.



Using Auto Range

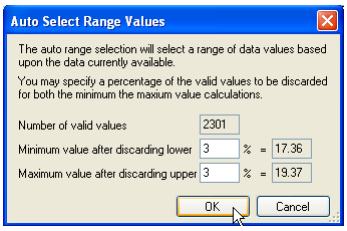
Auto range allows you to eliminate a percentage of the upper and lower ranges of all valid C_t values.

1 Click **Auto Range**.

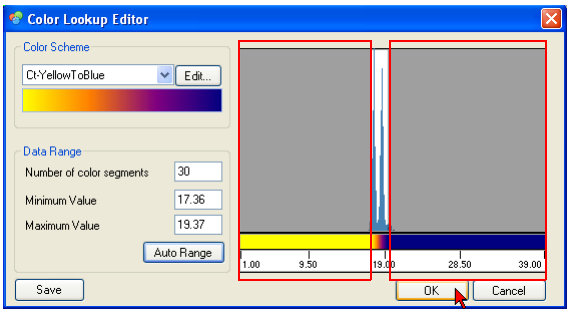


2 After reading the explanation in the Select Range Values dialog box, change values accordingly.

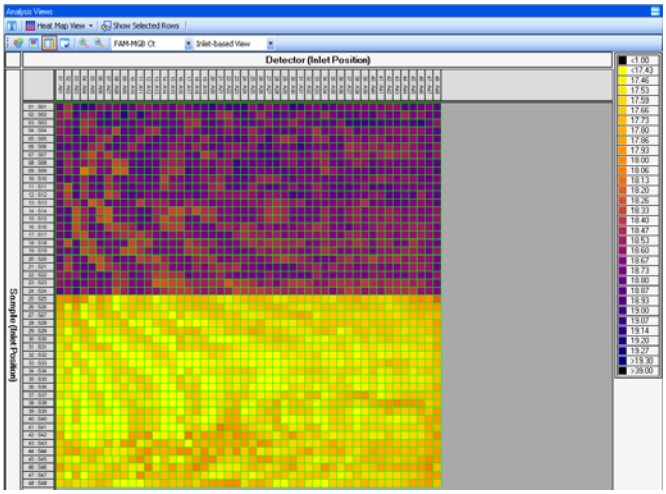
In the example below, the minimum and maximum values have been changed to 3. Therefore, after discarding the lower and upper 3% of valid C_t values, you are left with a range of 17.36 to 19.37.



This range is represented in the Color Lookup Editor illustrated below. Note the eliminated values (from 1 to 17.35, and 19.36 to 39) are now gray areas.



3 Click **OK** to see the changes in the heat map and the heat map legend as illustrated below.



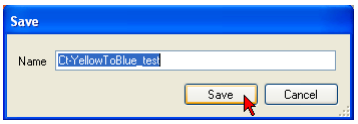
Saving Changes

To save custom parameters that you have set:

1 Click **Save**. The Save dialog opens.

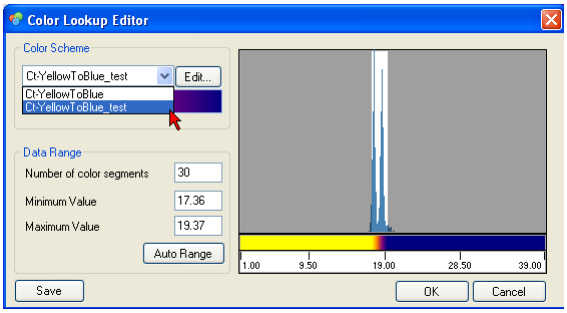
2 Type a name for your custom parameters.

3 Click **Save**.



The Color Lookup Editor opens.

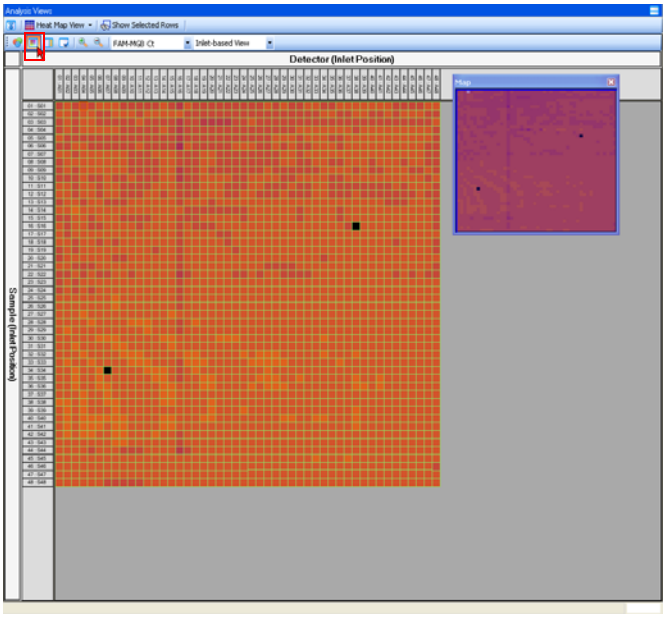
4 Click the Color Scheme drop-down menu to see the saved parameters.



Location Reference Map

Use the location map to reference your cell of interest within the entire framework of the chip.

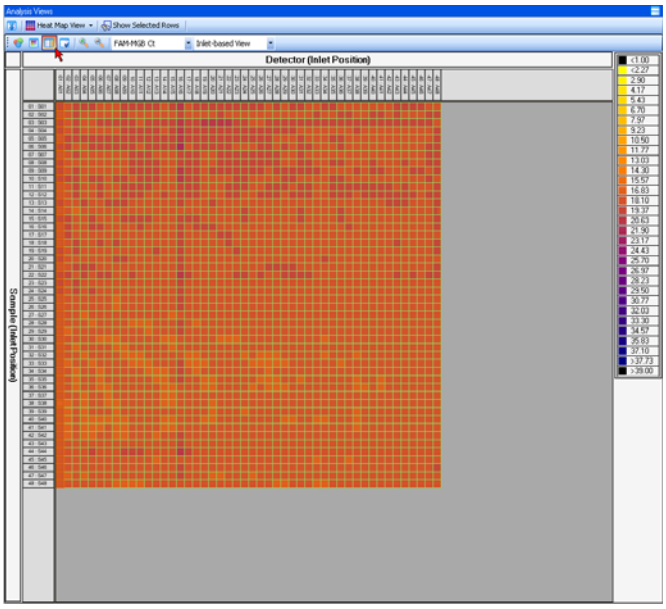
- Click the Location Reference map icon to open the map.



Legend

The legend is a color representation of the C values displayed on the heat map.

- Click the Legend icon to open the legend.



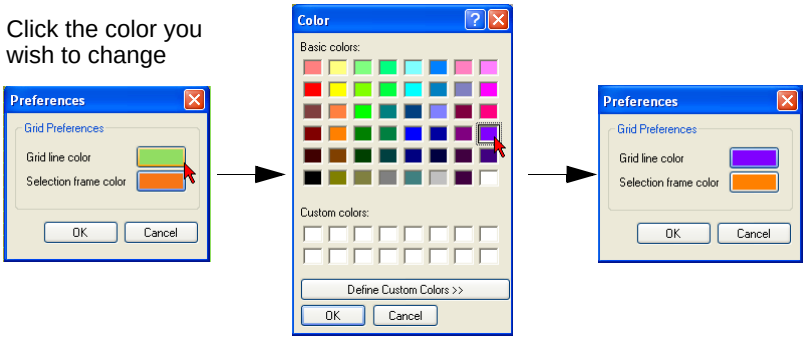
Preferences

Change the colors of the heat map grid lines and/or selected frame.

1 Click the Preferences icon.

2 In the Grid Preferences pane, click the color rectangle whose color you want to change.

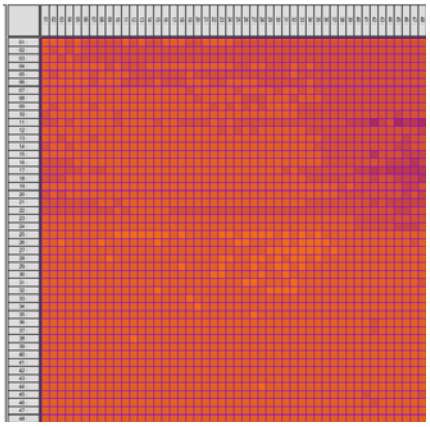
The color palette opens.



3 Click **OK**.

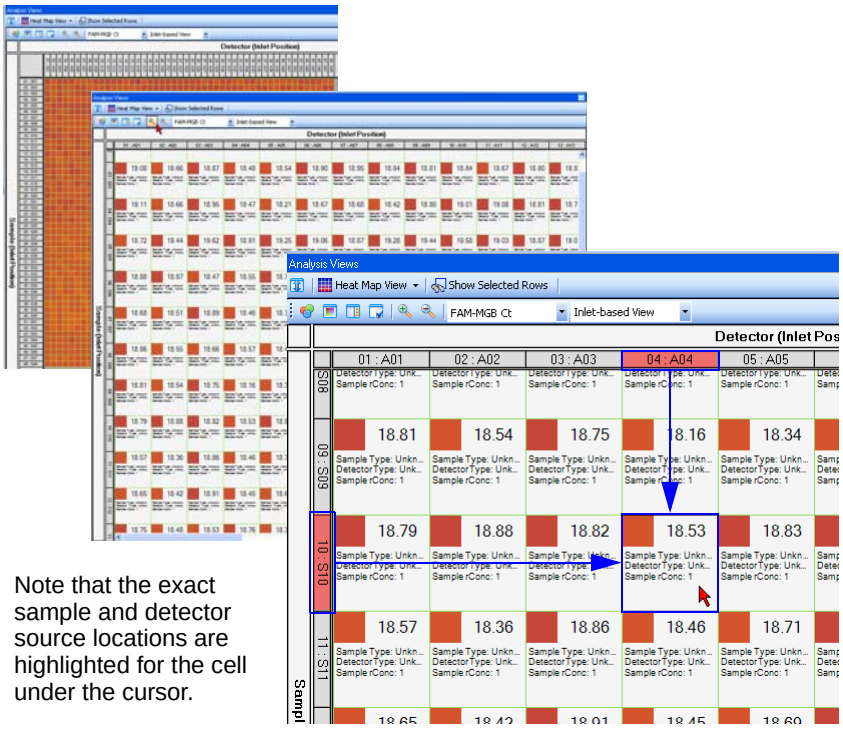
The purple grid lines display in the heat map.

NOTE: Perform the same procedure to change the frame color also.



Zoom

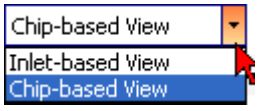
Increase or decrease the image view size by multi-clicking the magnifying glass icons (+ and –) .



Note that the exact sample and detector source locations are highlighted for the cell under the cursor.

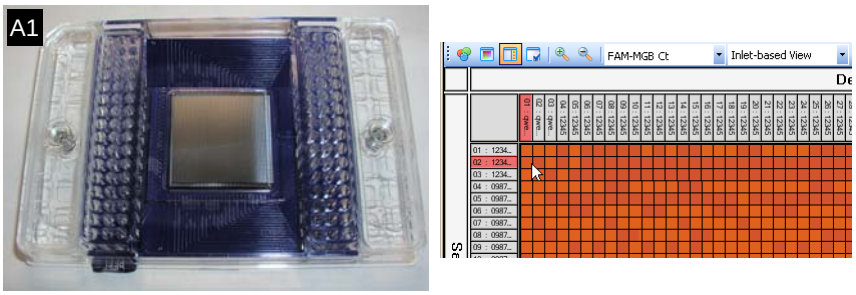
Inlet-based View/Chip-based View

Toggle between inlet-based and chip-based views in the Heat Map by selecting from the toolbar drop-down menu as shown below.



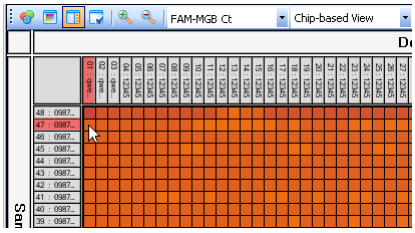
Inlet-based View

The inlet-based view shows the cell in the same numbered sequence as the inlets on the chip, as shown below.



Chip-based View

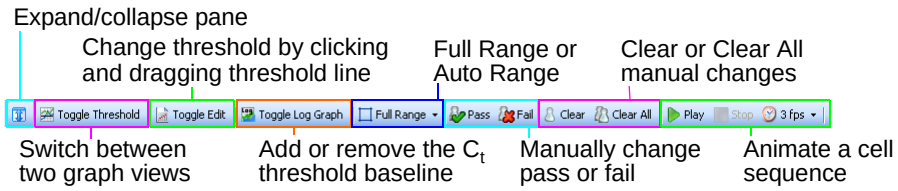
The chip-based view shows a sequence of numbers assigned to chambers on a chip counting from top-left corner, going left to right then top to bottom, as shown below.



Using the Graph View

View analyzed chip run data in two graph formats simultaneously: Raw and Amplification. In addition, play an animated data sequence.

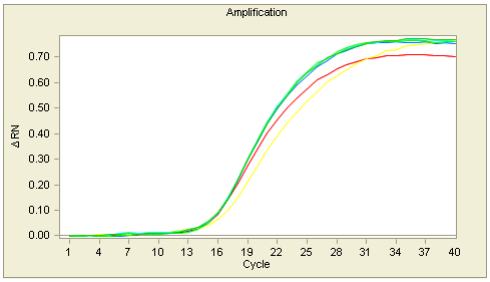
Graph Viewer Tool Bar



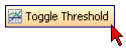
NOTE: Right-click within a graph window to change scale.

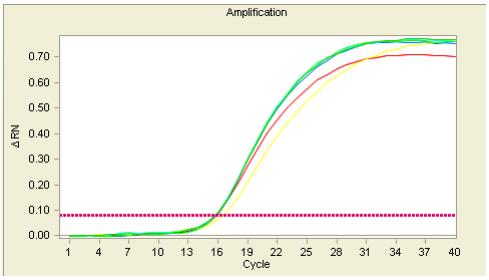
Toggle Threshold

Click this button to apply a C_t threshold line to the amplification graph as shown below.



Without threshold baseline

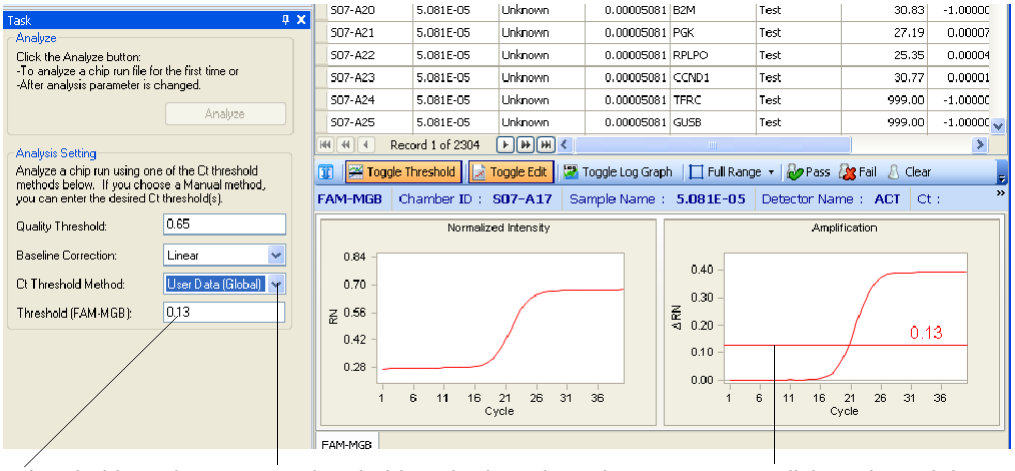




With threshold baseline

Toggle Edit

Click **Toggle Edit** in conjunction with Toggle Threshold enable moving the threshold bar to a new position by clicking and dragging it in the lower graph. This can only be done in User Data Global or User Data Detector threshold analysis methods.



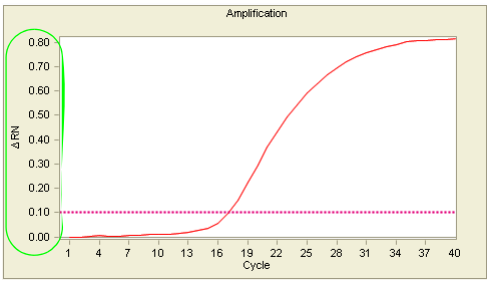
Threshold number changes as you re-position the threshold line.

C_t Threshold Method can be only User Data (Global) or User (Data Detectors) for Toggle Edit to be enabled.

Click on the red dot and drag the threshold bar to the desired position

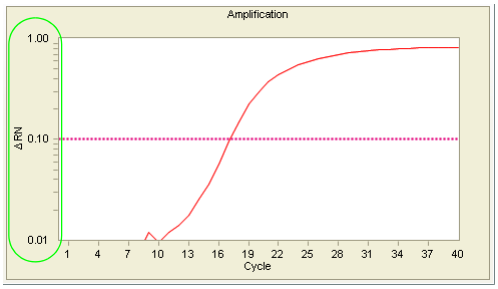
Toggle Log Graph

The Log graph shows more detail of the same view. Note the finer scale on the Log graph *y* axis below left.



Log graph *off*

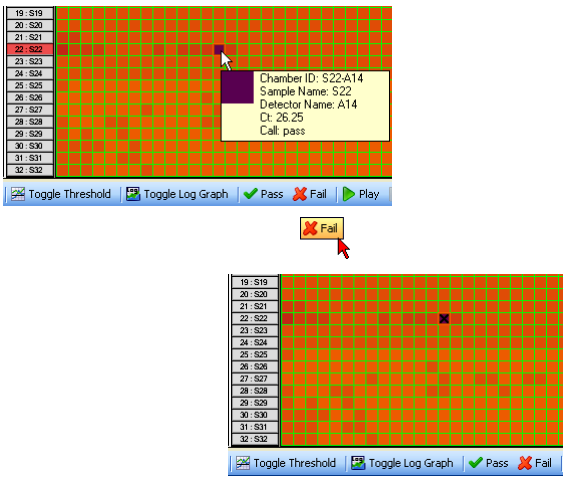
Log graph *on*



Changing Pass/Fail

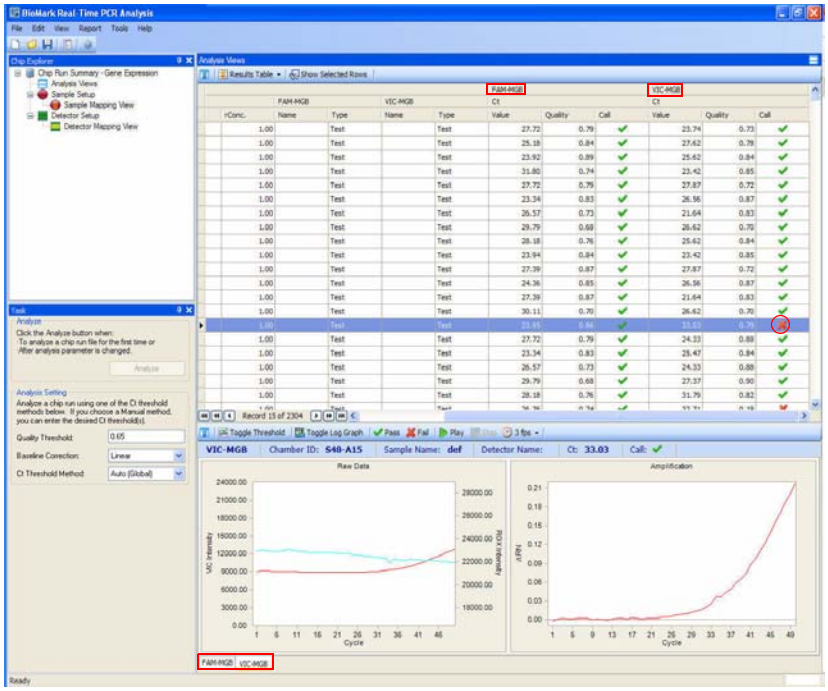
If the heat map reveals a problematic experiment, you can manually change the call to exclude the experiment. Change cells to pass or fail as appropriate. In the example below, the passing cell is manually failed.

1. Click a cell to activate it.
2. Click the **Pass** or the **Fail** icon. Or, click **Edit > Pass/Fail**.



If you used two probes, make sure the appropriate graph tab is active. In the example below, FAM-MGB and VIC-MGB probes each have a tab on the graph view. Click the appropriate graph tab before changing the call.

Probe-specific column heads



Probe-specific graph tabs

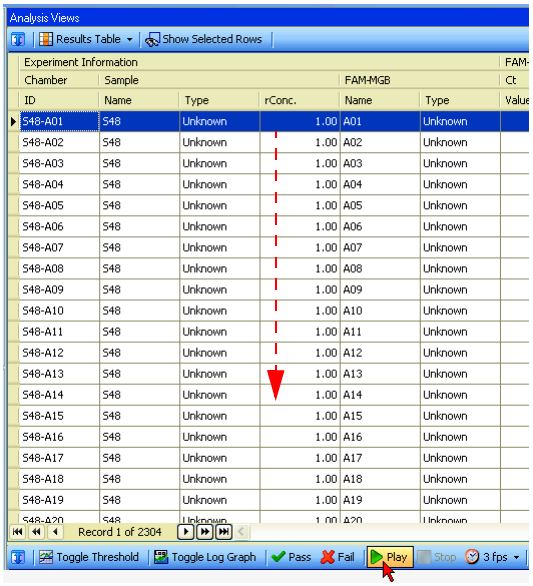
NOTE: You can use the Call Redo or Undo buttons to revert back to the original call state.

Using the Animate Feature

In the Graph Views, watch an animation of each cell on the entire chip in sequence. Use this feature while in the Results Table, Image View, and/or Heat Map.

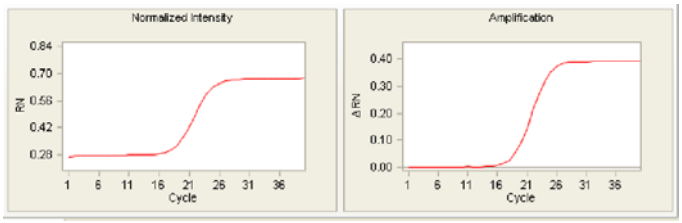
1. Click a cell or row.
2. Click **Play** Play.

   Watch the Normalized Intensity and Amplification graphs as each cell is displayed in sequence.



Click a row (in the Results Table in this example) and then click *Play*.

The highlighted row is displayed in the graph views and then the animation jumps to the next cell in sequence and so on until you click Stop or, the animation runs through the entire chip.

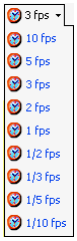


Each cell's data are displayed in the graphs during the animation.

3 (Optional) Adjust the animation speed.

a Click 3 fps .

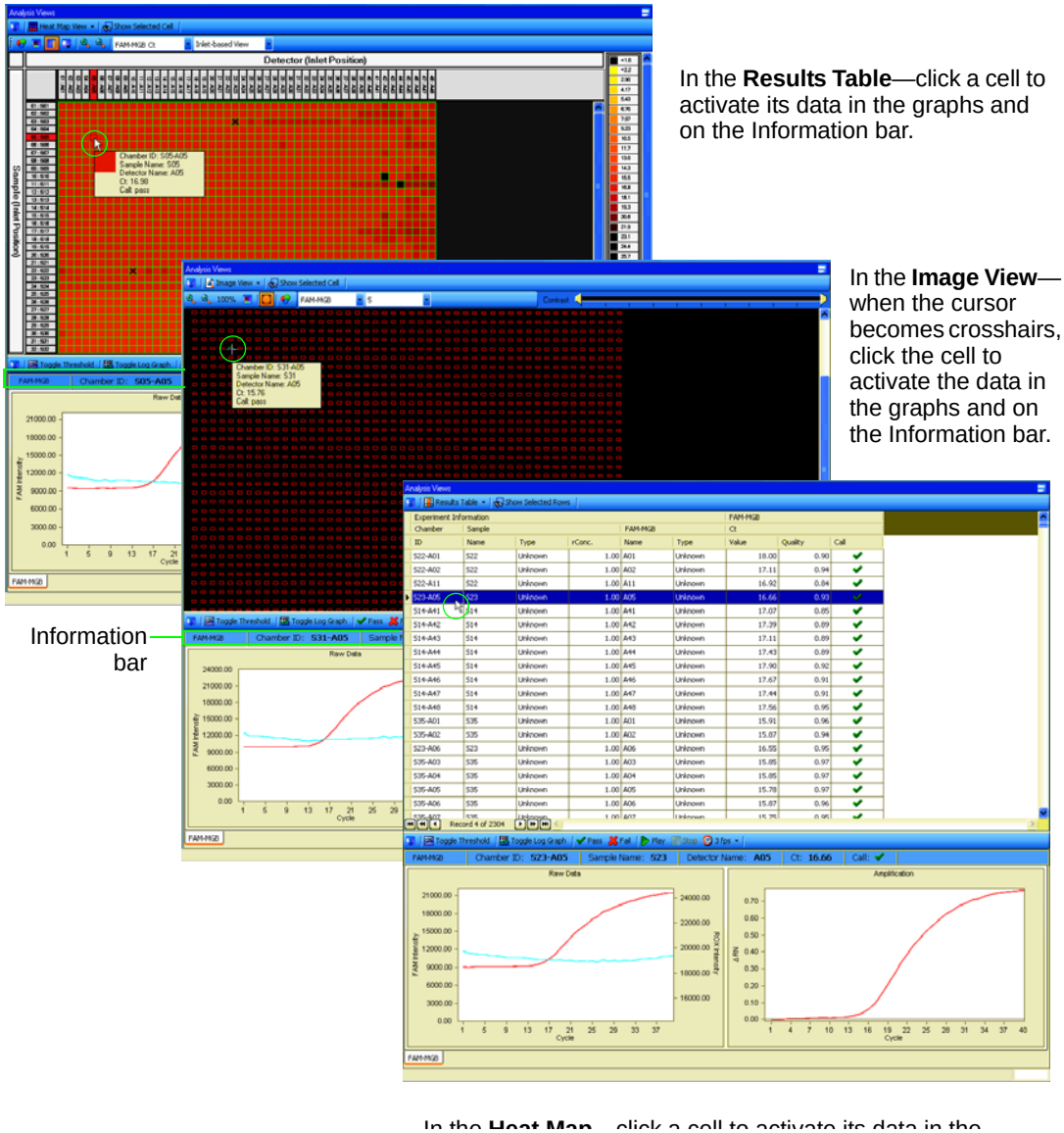
b Choose a viewing speed.



4 Click **Stop** Stop to stop the animation.

5 Click **Play** to continue the animation.

Selecting a Single Cell



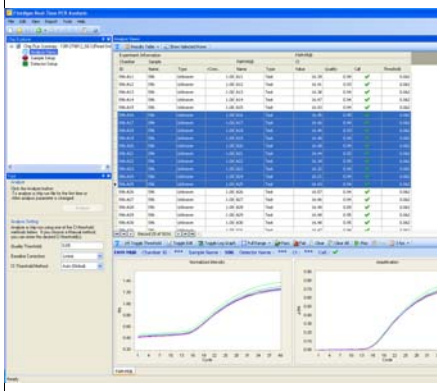
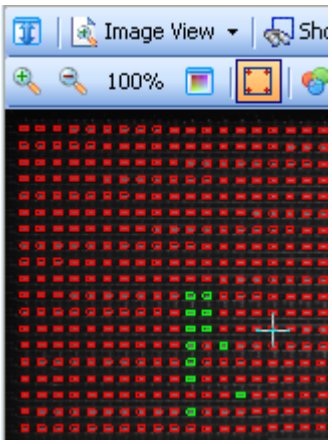
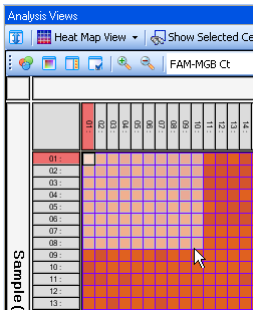
In the **Results Table**—click a cell to activate its data in the graphs and on the Information bar.

In the **Image View**—when the cursor becomes crosshairs, click the cell to activate the data in the graphs and on the Information bar.

In the **Heat Map**—click a cell to activate its data in the

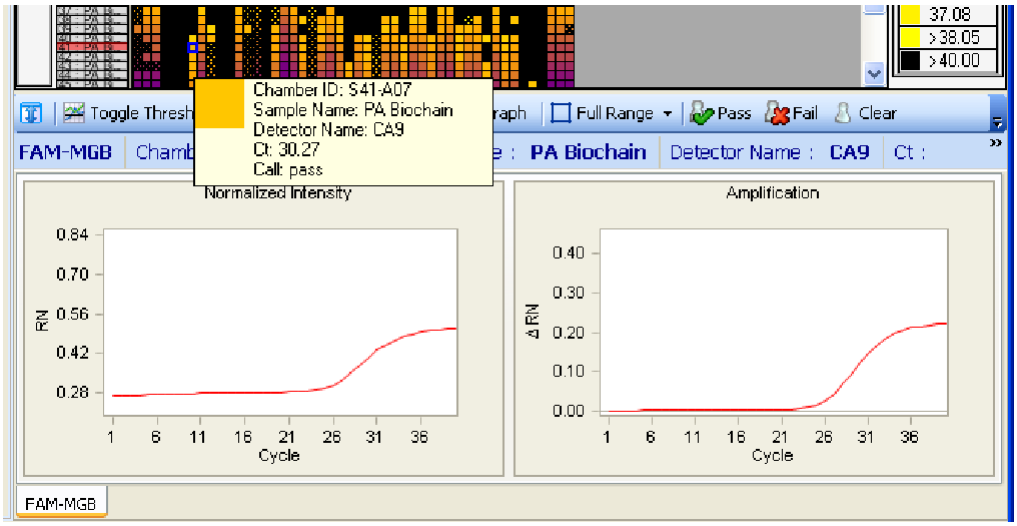
Selecting More Than One Cell

Isolate data for a single cell or for multiple cells in any analysis view (Results Table, Image View, or Heat Map) using the following methods

In the Analysis Views Window	Procedure	Example
The Results Table	Press and hold the keyboard **Shift** key and click the 2 outer cells for a continuous range of cells. The data for the range of cells display in the Graph Views. OR, Press and hold the keyboard **Ctrl** key while clicking individual cells.	
Image View	Press and hold the keyboard **Ctrl** key while clicking individual cells of interest. You cannot select a contiguous range in this view.	
Heat Map	Click a cell and then hold and drag to highlight a range of cells. OR, Press and hold the keyboard **Ctrl** key while clicking individual cells.	

Isolating Cell Data

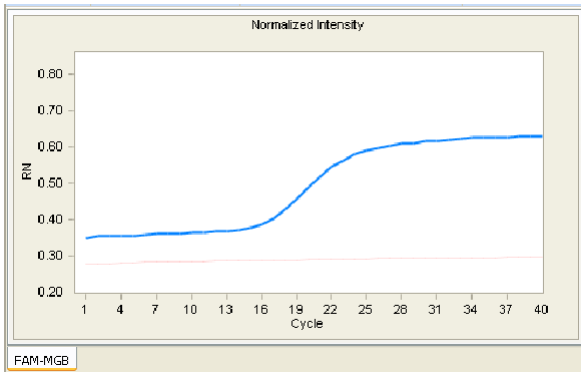
1 Click a sample cell (or cells) to display data in the Normalized Intensity and the Amplification graphs.



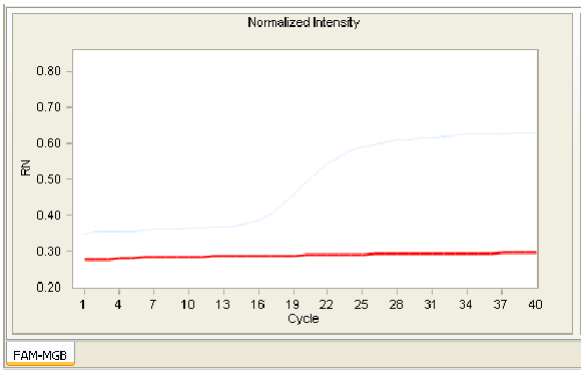
2 In either graph, hold the cursor over a sample of interest to highlight it.

NOTE: When the MGB line is highlighted, MGB intensity numbers are active while the FAM intensity numbers are inactive as shown in the graphic below. Conversely, click the FAM line and MGB intensity numbers become inactive.

MGB intensity highlighted



FAM intensity highlighted



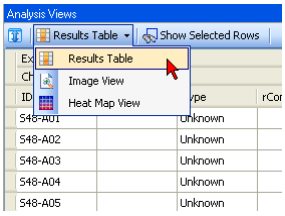
Exporting Data

You can export analysis data from the Heat Map and the Results Table views. The data are exported as Comma Separated Values (.csv) files that can be opened in Microsoft® Excel.

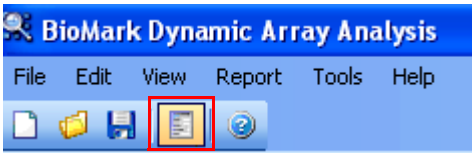
NOTE: The data look different in the .csv file depending on the view from which you exported.

Exporting Data from the Results Table

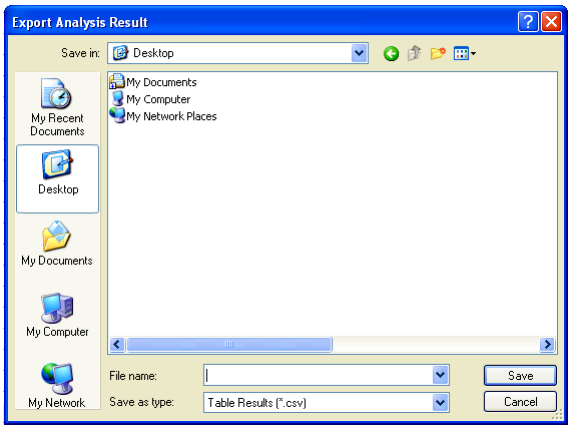
1 Make sure that you are in the Results Table window.



2 Click the Export icon (or **File > Export**).



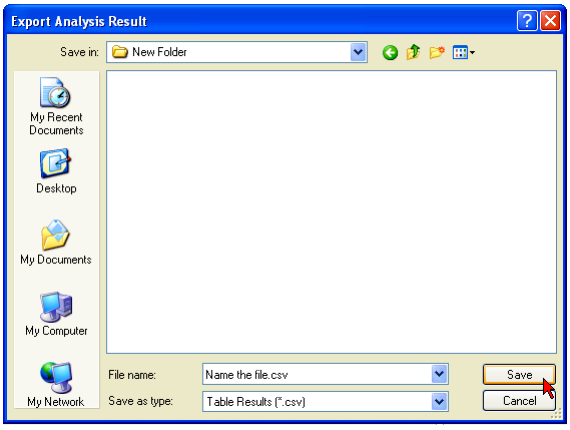
The Export Analysis Result dialog box opens.



3 Name the .csv file (that is, the data you are exporting).

4 Navigate to the save location using the Up One Level folder icon.

5 Click **Save** when you are at the save location.



Opening Exported Data (.csv files)

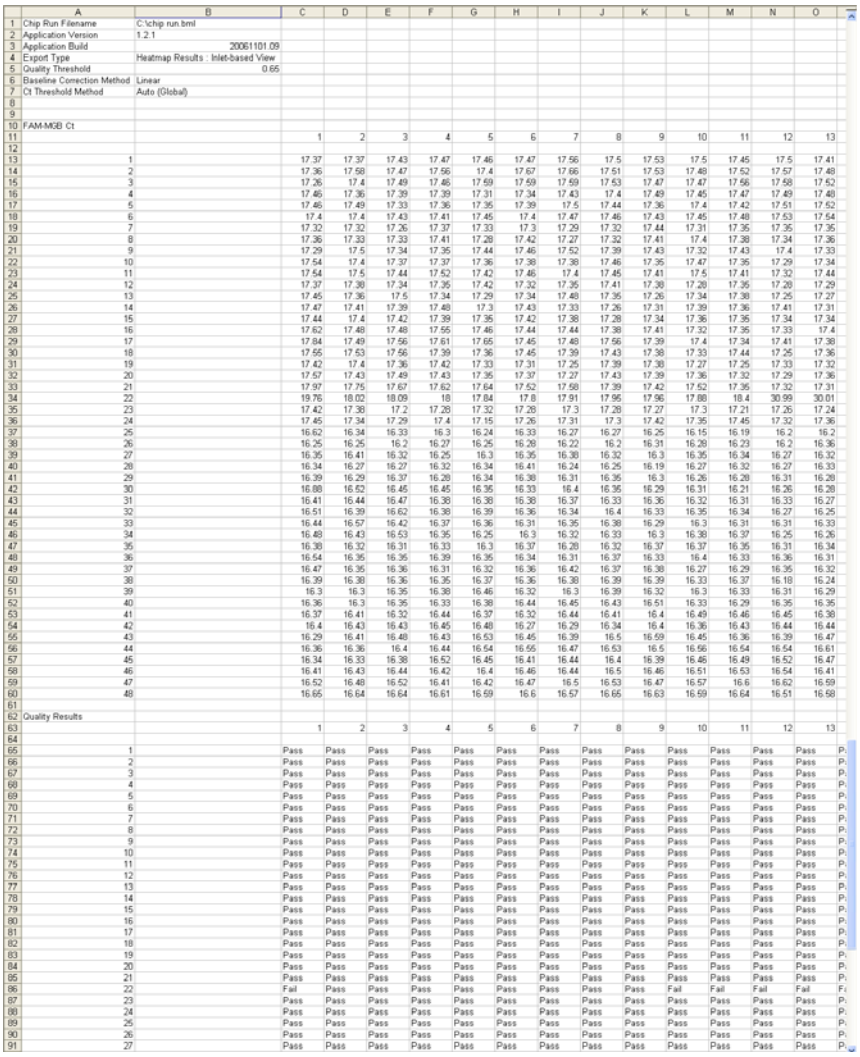
1 Double-click the saved .csv file of interest.

The exported .csv file below was saved from the Results Table view.

	A	B	C	D	E	F	G	H	I	J	K
1	Chip Run Filename	C:\Chip Run.bml									
2	Application Version	1.2.1									
3	Application Build	20061101.09									
4	Export Type	Table Results									
5	Quality Threshold	0.65									
6	Baseline Correction Method	Linear									
7	Ct Threshold Method	Auto (Global)									
8											
9											
10											
11	Chamber	Sample	Sample	Sample	FAM-MGB	FAM-MGB	Ct	Ct	Ct	Ct	
12	ID	Name	Type	rConc	Name	Type	Value	Quality	Call	Threshold	
13	S48-A01		Unknown	1		NRC	16.64704697	0.92	Pass	0.087195	
14	S48-A02		Unknown	1		Test	16.63592989	0.94	Pass	0.087195	
15	S48-A03		Unknown	1		Test	16.64165745	0.94	Pass	0.087195	
16	S48-A04		Unknown	1		Test	16.60683749	0.94	Pass	0.087195	
17	S48-A05		Unknown	1		Test	16.5905822	0.91	Pass	0.087195	
18	S48-A06		Unknown	1		Test	16.59913754	0.9	Pass	0.087195	
19	S48-A07		Unknown	1		Test	16.57397115	0.96	Pass	0.087195	
20	S48-A08		Unknown	1		Test	16.64511776	0.94	Pass	0.087195	
21	S48-A09		Unknown	1		Test	16.63198978	0.92	Pass	0.087195	
22	S48-A10		Unknown	1		Test	16.6896578	0.92	Pass	0.087195	
23	S48-A11		Unknown	1		Test	16.64416875	0.97	Pass	0.087195	
24	S48-A12		Unknown	1		Test	16.50895379	0.92	Pass	0.087195	
25	S48-A13		Unknown	1		Test	16.58137263	0.93	Pass	0.087195	
26	S48-A14		Unknown	1		Test	16.68415245	0.95	Pass	0.087195	
27	S48-A15		Unknown	1		Test	16.59621888	0.96	Pass	0.087195	
28	S48-A16		Unknown	1		Test	16.55044303	0.92	Pass	0.087195	
29	S48-A17		Unknown	1		Test	16.61231466	0.87	Pass	0.087195	
30	S48-A18		Unknown	1		Test	16.41596571	0.96	Pass	0.087195	
31	S48-A19		Unknown	1		Test	16.39270302	0.95	Pass	0.087195	
32	S48-A20		Unknown	1		Test	16.42114668	0.93	Pass	0.087195	
33	S48-A21		Unknown	1		Test	16.4339541	0.93	Pass	0.087195	
34	S48-A22		Unknown	1		Test	16.46969445	0.85	Pass	0.087195	
35	S48-A23		Unknown	1		Test	16.47757942	0.89	Pass	0.087195	
36	S48-A24		Unknown	1		Test	16.61660155	0.88	Pass	0.087195	
37	S48-A25		Unknown	1		Test	16.70991263	0.92	Pass	0.087195	
38	S48-A26		Unknown	1		Test	16.47624034	0.9	Pass	0.087195	
39	S48-A27		Unknown	1		Test	16.56970419	0.93	Pass	0.087195	
40	S48-A28		Unknown	1		Test	16.63766531	0.85	Pass	0.087195	
41	S48-A29		Unknown	1		Test	16.64719062	0.87	Pass	0.087195	
42	S48-A30		Unknown	1		Test	16.44827949	0.93	Pass	0.087195	
43	S48-A31		Unknown	1		Test	16.46781061	0.94	Pass	0.087195	
44	S48-A32		Unknown	1		Test	16.72249286	0.95	Pass	0.087195	
45	S48-A33		Unknown	1		Test	16.69084516	0.91	Pass	0.087195	
46	S48-A34		Unknown	1		Test	16.62098613	0.9	Pass	0.087195	

	A	B	C	D	E	F	G	H	I	J	K
2271	S22-A03		Unknown	1		Test	18.08691033	0.93	Pass	0.087195	
2272	S22-A04		Unknown	1		Test	17.99903398	0.92	Pass	0.087195	
2273	S22-A05		Unknown	1		Test	17.83982131	0.93	Pass	0.087195	
2274	S22-A06		Unknown	1		Test	17.80471292	0.94	Pass	0.087195	
2275	S22-A07		Unknown	1		Test	17.90847612	0.93	Pass	0.087195	
2276	S22-A08		Unknown	1		Test	17.95337336	0.93	Pass	0.087195	
2277	S22-A09		Unknown	1		Test	17.95888545	0.94	Pass	0.087195	
2278	S22-A10		Unknown	1		Test	17.88469637	0.46	Fail	0.087195	
2279	S22-A11		Unknown	1		Test	18.39513697	0.594738	Fail	0.087195	
2280	S22-A12		Unknown	1		Test	30.98544154	0.282696	Fail	0.087195	
2281	S22-A13		Unknown	1		Test	30.00733598	0.390302	Fail	0.087195	
2282	S22-A14		Unknown	1		Test	30.77122724	0.139323	Fail	0.087195	
2283	S22-A15		Unknown	1		Test	18.64205576	0.594643	Fail	0.087195	
2284	S22-A16		Unknown	1		Test	17.74144528	0.95	Pass	0.087195	
2285	S22-A17		Unknown	1		Test	17.75888218	0.96	Pass	0.087195	
2286	S22-A18		Unknown	1		Test	17.68173075	0.95	Pass	0.087195	
2287	S22-A19		Unknown	1		Test	17.67744264	0.97	Pass	0.087195	
2288	S22-A20		Unknown	1		Test	17.66148182	0.96	Pass	0.087195	
2289	S22-A21		Unknown	1		Test	17.5536887	0.96	Pass	0.087195	
2290	S22-A22		Unknown	1		Test	17.73049929	0.95	Pass	0.087195	
2291	S22-A23		Unknown	1		Test	17.74766732	0.92	Pass	0.087195	
2292	S22-A24		Unknown	1		Test	17.62494516	0.96	Pass	0.087195	
2293	S22-A25		Unknown	1		Test	17.58283209	0.96	Pass	0.087195	
2294	S22-A26		Unknown	1		Test	17.59893934	0.94	Pass	0.087195	
2295	S22-A27		Unknown	1		Test	17.64820926	0.95	Pass	0.087195	
2296	S22-A28		Unknown	1		Test	17.66065755	0.95	Pass	0.087195	
2297	S22-A29		Unknown	1		Test	17.71605798	0.95	Pass	0.087195	
2298	S22-A30		Unknown	1		Test	17.65024087	0.95	Pass	0.087195	
2299	S22-A31		Unknown	1		Test	17.72965224	0.95	Pass	0.087195	
2300	S22-A32		Unknown	1		Test	17.84150677	0.93	Pass	0.087195	
2301	S22-A33		Unknown	1		Test	17.68156005	0.94	Pass	0.087195	
2302	S22-A34		Unknown	1		Test	17.68869201	0.95	Pass	0.087195	
2303	S22-A35		Unknown	1		Test	17.85066492	0.95	Pass	0.087195	
2304	S22-A36		Unknown	1		Test	17.8610457	0.93	Pass	0.087195	
2305	S22-A37		Unknown	1		Test	17.91054909	0.95	Pass	0.087195	
2306	S22-A38		Unknown	1		Test	18.00792537	0.96	Pass	0.087195	
2307	S22-A39		Unknown	1		Test	17.93665695	0.92	Pass	0.087195	
2308	S22-A40		Unknown	1		Test	18.00871712	0.95	Pass	0.087195	
2309	S22-A41		Unknown	1		Test	18.23584288	0.9	Pass	0.087195	
2310	S22-A42		Unknown	1		Test	18.67539952	0.91	Pass	0.087195	
2311	S22-A43		Unknown	1		Test	18.38322824	0.94	Pass	0.087195	
2312	S22-A44		Unknown	1		Test	18.48385076	0.9	Pass	0.087195	
2313	S22-A45		Unknown	1		Test	19.19560089	0.778696	Pass	0.087195	
2314	S22-A46		Unknown	1		Test	19.14121213	0.744293	Pass	0.087195	
2315	S22-A47		Unknown	1		Test	19.24169333	0.589493	Fail	0.087195	
2316	S22-A48		Unknown	1		Test	999	0	Fail	0.087195	

The exported .csv file below was saved from the Heat Map view.

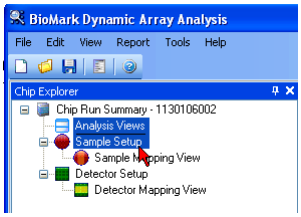


	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	Chip Run Filename	C:\chip run.bml													
2	Application Version	1.2.1													
3	Application Build	20061101.09													
4	Export Type	Heatmap Results : Inlet-based View													
5	Quality Threshold	0.65													
6	Baseline Correction Method	Linear													
7	Ct Threshold Method	Auto (Global)													
8															
9															
10	FAM-MGB Ct														
11			1	2	3	4	5	6	7	8	9	10	11	12	13
12															
13	1		17.37	17.37	17.43	17.47	17.46	17.47	17.56	17.5	17.53	17.5	17.45	17.5	17.41
14	2		17.36	17.58	17.47	17.56	17.4	17.67	17.66	17.51	17.53	17.48	17.52	17.57	17.48
15	3		17.26	17.4	17.49	17.46	17.69	17.69	17.69	17.53	17.47	17.47	17.66	17.58	17.52
16	4		17.46	17.36	17.39	17.39	17.31	17.34	17.43	17.4	17.49	17.45	17.47	17.49	17.48
17	5		17.46	17.49	17.33	17.36	17.35	17.39	17.5	17.44	17.36	17.4	17.42	17.51	17.52
18	6		17.4	17.4	17.43	17.41	17.45	17.4	17.47	17.46	17.43	17.45	17.48	17.53	17.54
19	7		17.32	17.32	17.26	17.37	17.33	17.3	17.29	17.32	17.44	17.31	17.35	17.35	17.35
20	8		17.36	17.33	17.33	17.41	17.28	17.42	17.27	17.32	17.41	17.4	17.38	17.34	17.36
21	9		17.29	17.5	17.34	17.35	17.44	17.46	17.52	17.39	17.43	17.32	17.43	17.4	17.33
22	10		17.54	17.4	17.37	17.37	17.36	17.38	17.38	17.46	17.35	17.47	17.35	17.29	17.34
23	11		17.54	17.5	17.44	17.52	17.42	17.46	17.4	17.45	17.41	17.5	17.41	17.32	17.44
24	12		17.37	17.38	17.34	17.35	17.42	17.32	17.35	17.41	17.38	17.28	17.35	17.28	17.29
25	13		17.45	17.36	17.5	17.34	17.29	17.34	17.48	17.35	17.26	17.34	17.38	17.25	17.27
26	14		17.47	17.41	17.39	17.49	17.3	17.43	17.33	17.26	17.31	17.39	17.36	17.41	17.31
27	15		17.44	17.4	17.42	17.39	17.35	17.42	17.38	17.28	17.34	17.36	17.35	17.34	17.34
28	16		17.62	17.48	17.48	17.55	17.46	17.44	17.44	17.38	17.41	17.32	17.35	17.33	17.4
29	17		17.84	17.49	17.56	17.61	17.65	17.45	17.48	17.56	17.39	17.4	17.34	17.41	17.38
30	18		17.55	17.53	17.56	17.39	17.36	17.45	17.39	17.43	17.38	17.33	17.44	17.25	17.36
31	19		17.42	17.4	17.36	17.42	17.33	17.31	17.25	17.39	17.38	17.27	17.25	17.33	17.32
32	20		17.57	17.43	17.49	17.43	17.35	17.37	17.27	17.43	17.39	17.36	17.32	17.29	17.36
33	21		17.97	17.75	17.67	17.62	17.64	17.52	17.58	17.39	17.42	17.52	17.35	17.32	17.31
34	22		19.76	18.02	18.09	18	17.84	17.8	17.91	17.96	17.96	17.88	18.4	30.99	30.01
35	23		17.42	17.38	17.2	17.28	17.32	17.28	17.3	17.28	17.27	17.3	17.21	17.26	17.24
36	24		17.45	17.34	17.29	17.4	17.15	17.26	17.31	17.3	17.42	17.35	17.45	17.32	17.36
37	25		16.62	16.34	16.33	16.3	16.24	16.33	16.27	16.27	16.25	16.15	16.19	16.2	16.2
38	26		16.25	16.25	16.2	16.27	16.25	16.28	16.22	16.2	16.31	16.28	16.23	16.2	16.36
39	27		16.35	16.41	16.32	16.25	16.3	16.35	16.38	16.32	16.3	16.35	16.34	16.27	16.32
40	28		16.34	16.27	16.27	16.32	16.34	16.41	16.24	16.25	16.19	16.27	16.32	16.27	16.33
41	29		16.39	16.29	16.37	16.28	16.34	16.38	16.31	16.35	16.3	16.26	16.28	16.31	16.28
42	30		16.88	16.62	16.45	16.45	16.35	16.33	16.4	16.35	16.29	16.31	16.21	16.26	16.28
43	31		16.41	16.44	16.47	16.36	16.36	16.38	16.37	16.33	16.36	16.32	16.31	16.33	16.27
44	32		16.51	16.39	16.62	16.38	16.39	16.36	16.34	16.4	16.33	16.35	16.34	16.27	16.25
45	33		16.44	16.57	16.42	16.37	16.36	16.31	16.35	16.38	16.29	16.3	16.31	16.31	16.33
46	34		16.48	16.43	16.53	16.35	16.25	16.3	16.32	16.33	16.3	16.38	16.37	16.25	16.26
47	35		16.38	16.32	16.31	16.33	16.3	16.37	16.28	16.32	16.37	16.37	16.35	16.31	16.34
48	36		16.54	16.35	16.35	16.39	16.35	16.34	16.31	16.37	16.33	16.4	16.33	16.36	16.31
49	37		16.47	16.35	16.36	16.31	16.32	16.36	16.42	16.37	16.38	16.27	16.29	16.35	16.32
50	38		16.39	16.38	16.36	16.35	16.37	16.36	16.38	16.39	16.39	16.33	16.37	16.18	16.24
51	39		16.3	16.3	16.35	16.38	16.46	16.32	16.3	16.39	16.32	16.3	16.33	16.31	16.29
52	40		16.36	16.3	16.35	16.33	16.38	16.44	16.45	16.43	16.51	16.33	16.29	16.35	16.35
53	41		16.37	16.41	16.32	16.44	16.37	16.32	16.44	16.41	16.4	16.49	16.46	16.45	16.38
54	42		16.4	16.43	16.43	16.45	16.48	16.27	16.29	16.34	16.4	16.36	16.43	16.44	16.44
55	43		16.29	16.41	16.48	16.43	16.53	16.45	16.39	16.5	16.59	16.45	16.36	16.39	16.47
56	44		16.36	16.36	16.4	16.44	16.54	16.55	16.47	16.53	16.5	16.56	16.54	16.54	16.61
57	45		16.34	16.33	16.38	16.52	16.45	16.41	16.44	16.4	16.39	16.46	16.49	16.52	16.47
58	46		16.41	16.43	16.44	16.42	16.4	16.46	16.44	16.5	16.46	16.51	16.53	16.54	16.41
59	47		16.52	16.48	16.52	16.41	16.42	16.47	16.5	16.53	16.47	16.57	16.6	16.62	16.59
60	48		16.65	16.64	16.64	16.61	16.59	16.6	16.57	16.65	16.63	16.59	16.64	16.51	16.58
61															
62	Quality Results														
63			1	2	3	4	5	6	7	8	9	10	11	12	13
64															
65	1		Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
66	2		Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
67	3		Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
68	4		Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
69	5		Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
70	6		Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
71	7		Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
72	8		Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
73	9		Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
74	10		Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
75	11		Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
76	12		Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
77	13		Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
78	14		Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
79	15		Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
80	16		Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
81	17		Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
82	18		Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
83	19		Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
84	20		Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
85	21		Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
86	22		Fail	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Fail	Fail	Fail	Fail
87	23		Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
88	24		Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
89	25		Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
90	26		Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
91	27		Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass

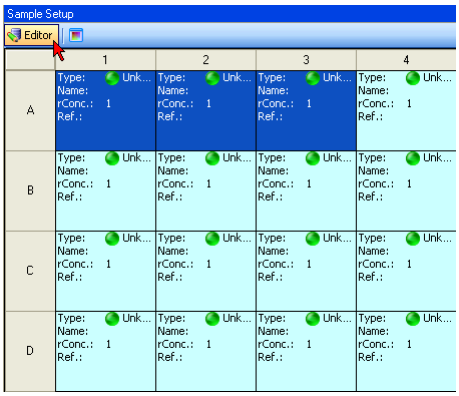
Calculating Delta C_t Sample Values

To calculate the delta (Δ) C_t samples:

1 Click **Sample Setup**.

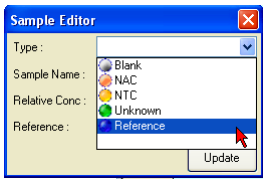


2 Select 1 to 3 cells where the reference sample was added.



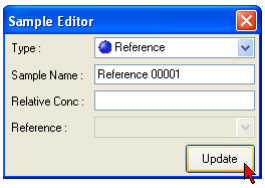
3 Click **Editor** to open the Sample Editor.

4 Click **Reference** in the Type drop-down menu.

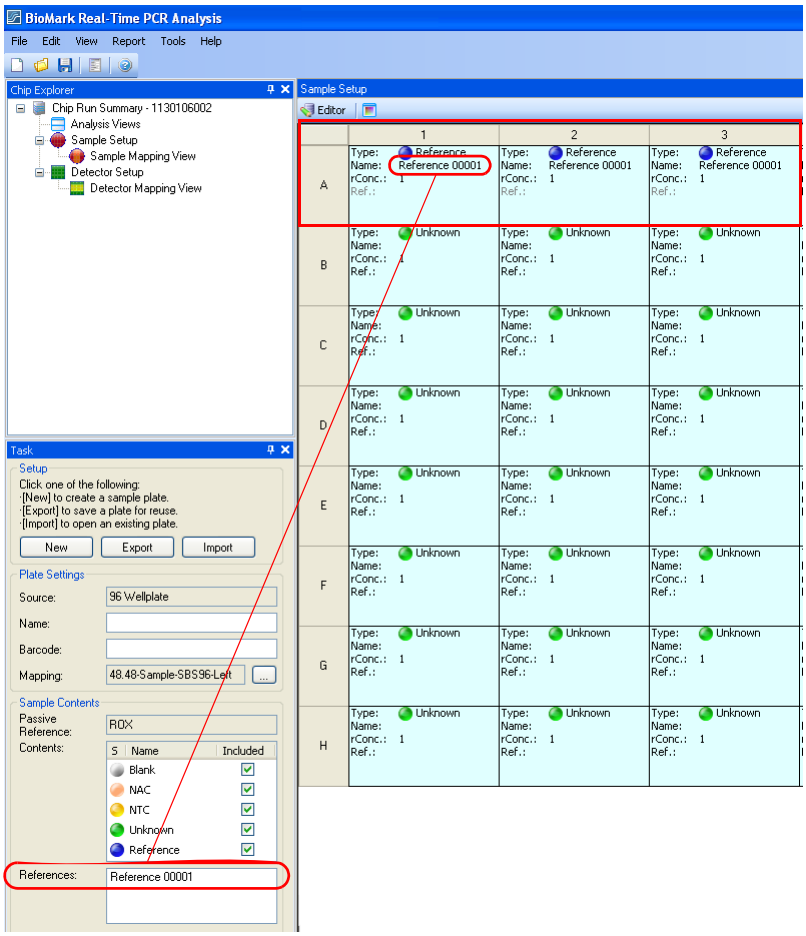


5 Enter a name in the Sample Name text box.

6 Click **Update**.



The Sample Setup reflects the change.


BioMark Real-Time PCR Analysis
File Edit View Report Tools Help
Chip Explorer
Chip Run Summary - 1130106002
Analysis Views
Sample Setup
Sample Mapping View
Detector Setup
Detector Mapping View
Task
Setup
Click one of the following:
-[New] to create a sample plate.
-[Export] to save a plate for reuse.
-[Import] to open an existing plate.
New
Export
Import
Plate Settings
Source:
96 Wellplate
Name:
Barcode:
Mapping:
48.48-Sample-SBS96-Left
Sample Contents
Passive Reference:
ROX
Contents:
S
Name
Included
Blank
NAC
NTC
Unknown
Reference
References:
Reference 00001
Sample Setup
Editor
1
2
3
A
B
C
D
E
F
G
H
Type: Reference
Name: Reference 00001
rConc.: 1
Ref.:
Type: Unknown
Name:
rConc.: 1
Ref.:

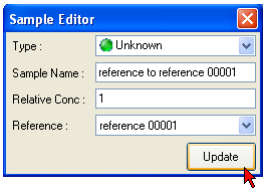
7 Select all cells that you want to reference. Typically, you select all the cells (except for the three reference cells) as in the example below.

8 Click **Editor**.

9 Enter a sample name.

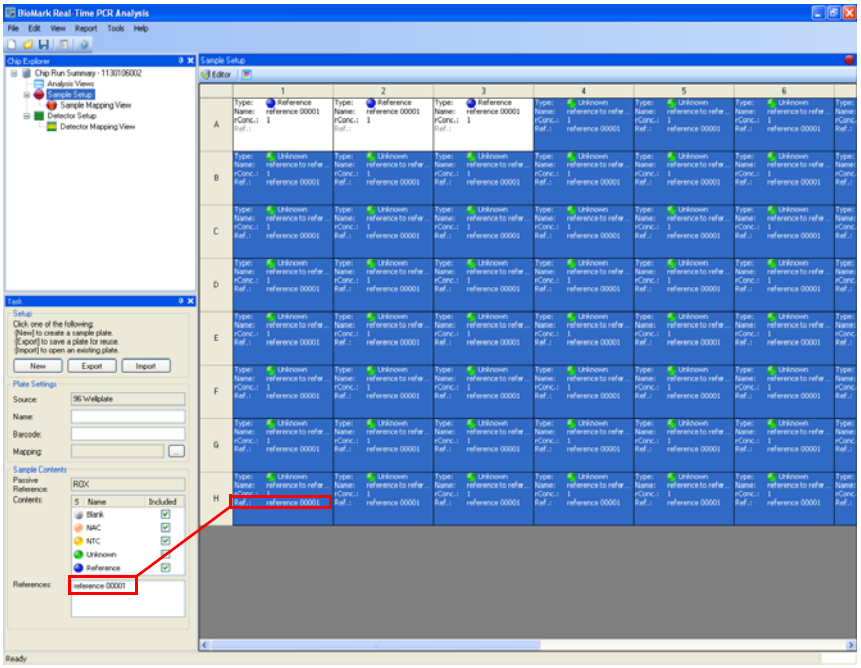
10 Select **Unknown** from the Type drop-down menu.

11 Select the reference you created from the Reference drop-down menu.



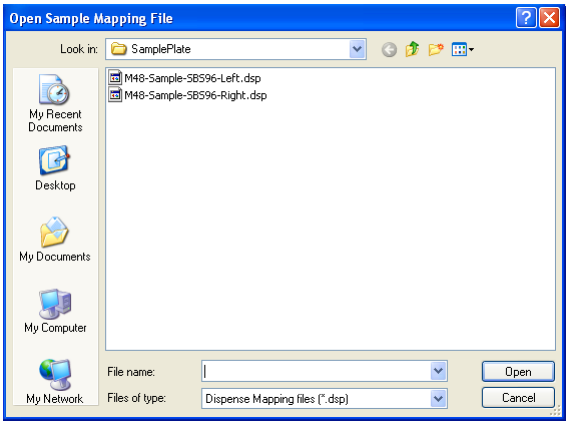
12 Click **Update**.

The changes are recorded as shown in the example below.



13 Click the mapping icon.

The Open Sample Mapping File dialog box opens.

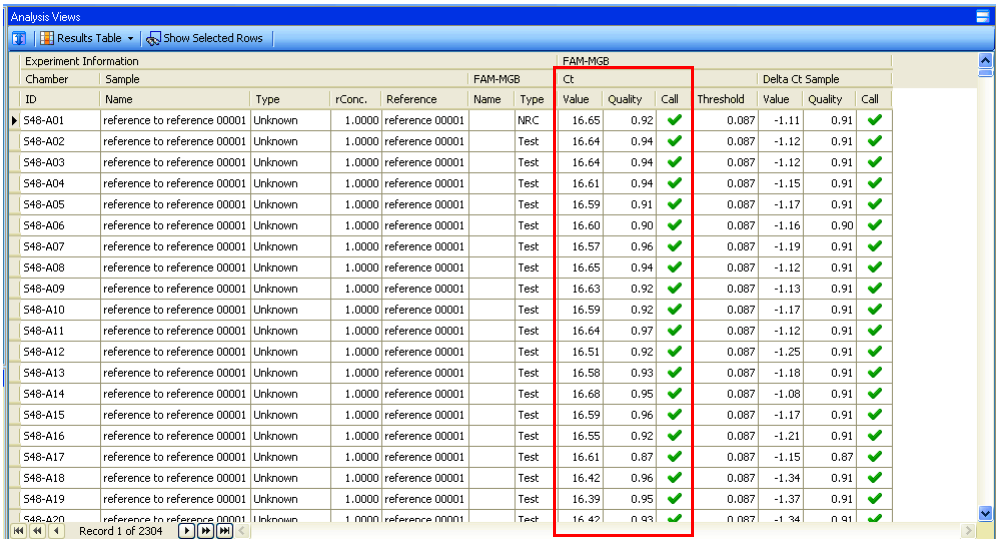


14 Double-click left or right mapping.

15 Click **Analysis Views**.

16 Click **Analyze**.

ΔC_t sample values are now available in the Results Table view.

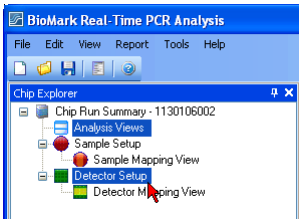


Calculating Delta C_t Detector Values

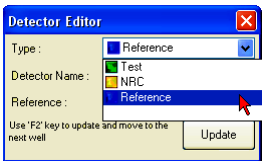
To calculate ΔC_t detector values, follow the procedure described in the previous section, "Calculating Delta Ct Sample Values" on page 110.

Steps that are specific to the procedure for calculating ΔC_t detector values are described below.

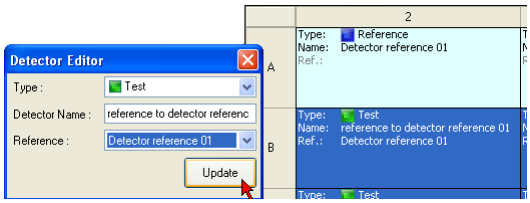
- At Step 1 on page 110, click **Detector Setup**.



- At Step 4 on page 110, click **Reference** in the Detector Editor.



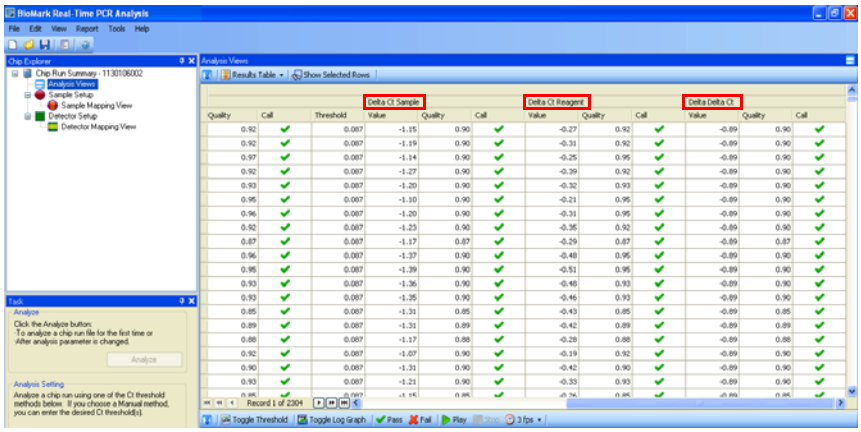
- At Step 10 on page 112, click **Test** in the in the Type drop-down menu.



Delta-Delta C_t Values

The $\Delta\Delta C_t$ values are available to you after the sample and the detector ΔC_t values are calculated (see "Calculating Delta Ct Sample Values" on page 110 and, "Calculating Delta Ct Detector Values" on page 114).

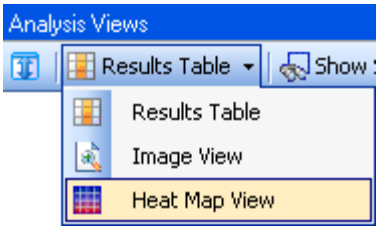
When sample and detector ΔC_t values have been calculated, click Analysis Views to see $\Delta\Delta C_t$ data:



Viewing Delta C_t Data in the Heat Map

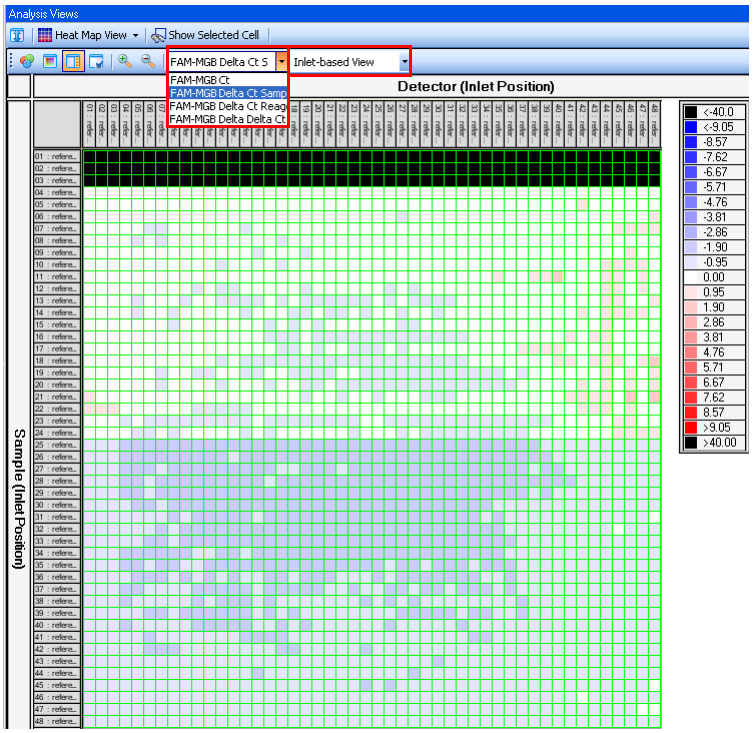
In addition to viewing ΔC_t and $\Delta\Delta C_t$ data in the Results Table, view ΔC_t data in the Heat Map.

1. Click **Analysis View**.
2. Click **Analyze**, if necessary.
3. Click **Heat Map View** from the Results Table drop-down menu.



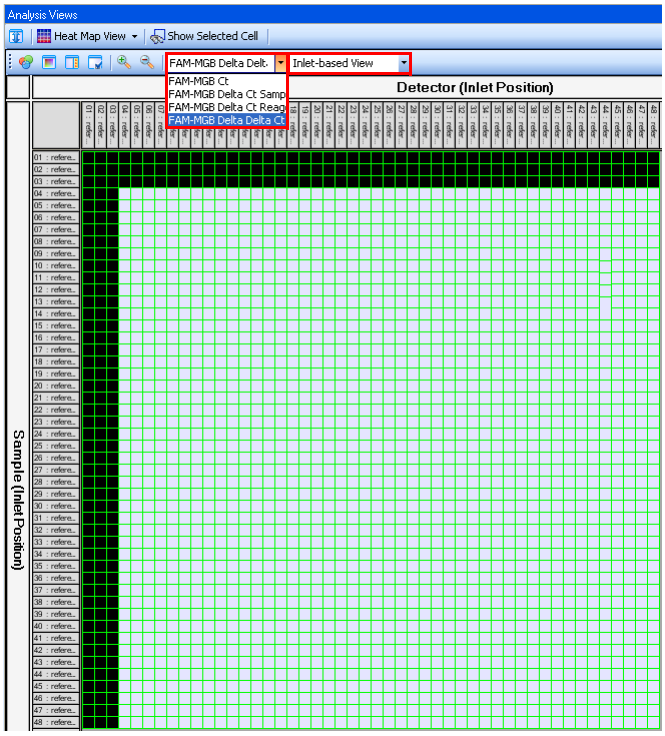
4 Select **FAM-MGB Delta C_t Sample** from the drop-down menu.

Reference cells:



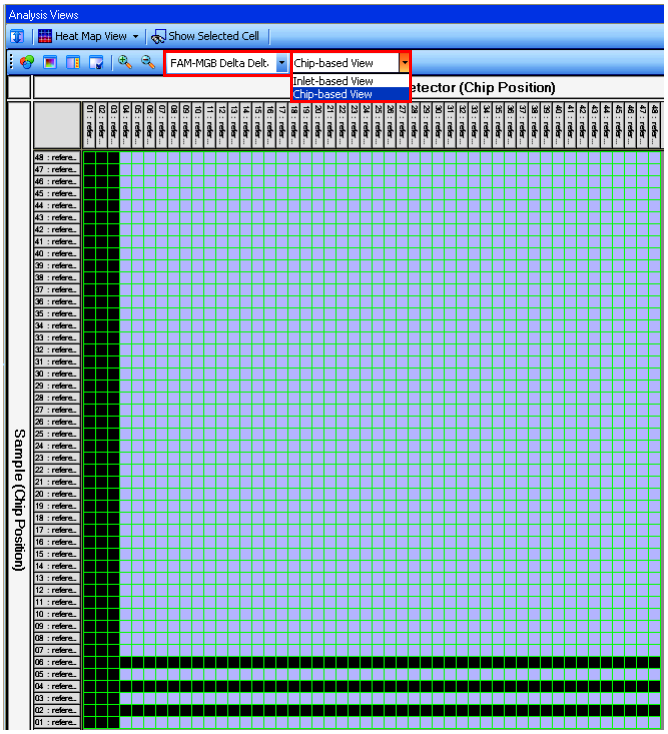
FAM-MGB Delta-Delta C_t Heat Map Data with Inlet-based View

Reference cells:



FAM-MGB Delta-Delta C_t Heat Map Data with a Chip-based View

Reference cells:



Congratulations, you have successfully viewed your analyzed chip run data. Proceed to the appendix for information on the install test.

Opening a View-Only Genotyping Chip Run

You may open a genotyping chip run to change analysis parameters, re-analyze the data, and export the results. However, you cannot save the open chip run.

Viewing Chip Run Data in the Calibration Curve View

4

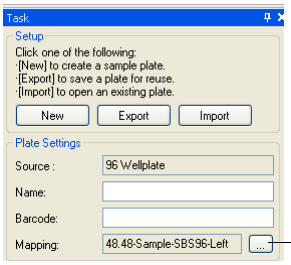
In this chapter:

Introduction

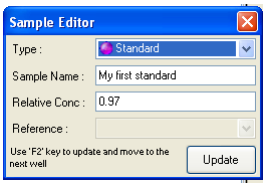
The Calibration Curve View Module (CCVM) (also known as “standard curve”) is a view that allows the user to create calibration curves based on the C_t and known concentration differences of samples on the chip. After calibration curves are created, they are used to determine the approximate concentration of unknown samples on the chip. The approximate values are displayed in a table format.

For CCVM to appear in the BioMark Real-Time PCR Analysis software, you must:

1. Open an unanalyzed chip run. See “Opening an Existing Chip Run” on page 29 for more information.
2. Select **Analysis Views** in the Chip Explorer pane.
3. Click the **Analyze** button.
4. Select **Sample Setup** in the Chip Explorer pane.
5. Click **New** to set up a new sample plate. Choose **SBS plate** or **Sample Inlet** for your container type. (For more detail see “Setting Up a Sample Plate” on page 33.)
6. Select a Mapping option (left- or right-side maps).



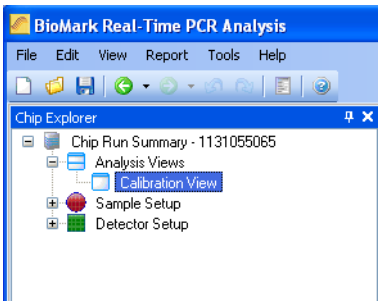
7. Use the **Editor** to annotate your sample cells. Make sure at least two wells are of the **Standard** type (essential for CCVM) and are named and have concentration values.



8. Set up Detector plate. Fore more detailed information see “Setting up a Detector Plate” on page 45.
9. Select **Analysis Views** in the Chip Explorer pane.
10. Click the **Analyze** button.

    The Analysis Views item should now have a plus sign infront of it in the Chip Explorer pane.

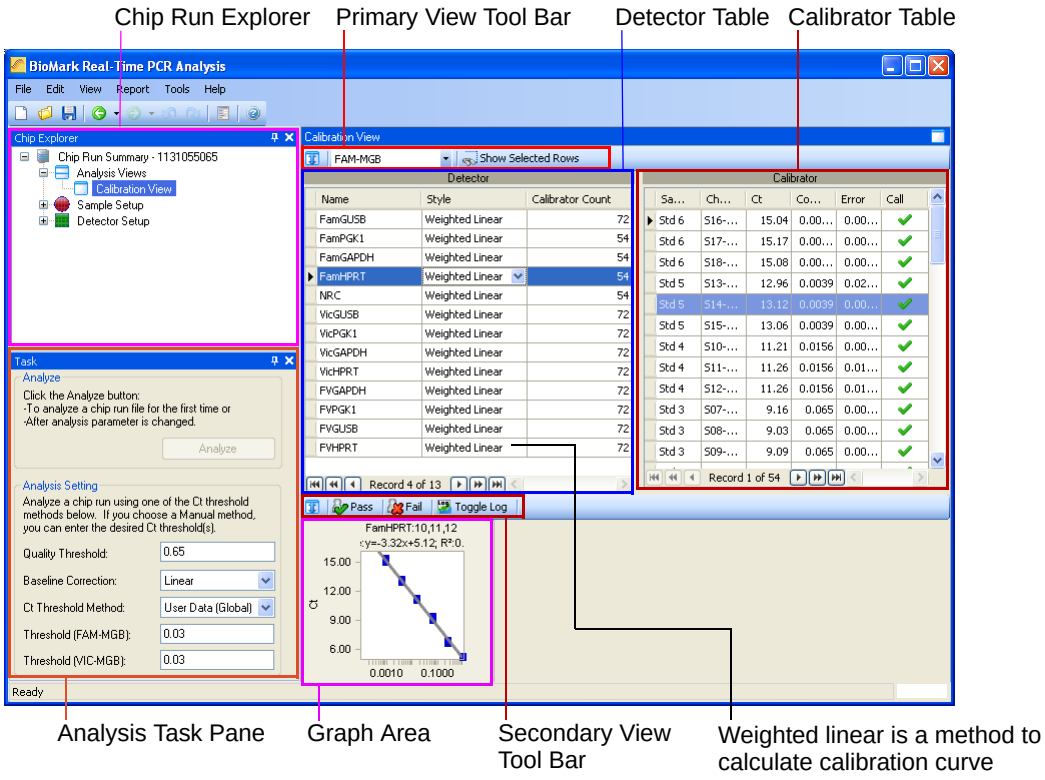
11 Expand the plus sign (+) next to Analysis Views and the **Calibration View** option appears on the tree.



12 Select **Calibration View** to launch the CCVM page.

CCVM Page Example

Below is the CCVM page, which consists of five individual panes and two tool bars.



Using CCVM to Determine Concentration Levels of Unknown Samples

The objective of using CCVM, is to set up a chip run with at least two standard type wells (where the concentration of DNA is known) and to predict the concentration of the unknown type samples. Four to six standard type samples are recommended. The calls of experiments that include standard samples are then plotted on the graph pane.

CCVM also allows you to modify calls associated with calibrators. This action is the same as modifying calls in the other views, but also has effect of adding or removing datapoints from the regression line (calibration curve) calculation.

When first launched, CCVM displays the contents and calibration curves of the data. These curves are created with a default fitting method (weighted linear).

1 In the Primary View Tool Bar, select a probe type, such as FAM MGB.

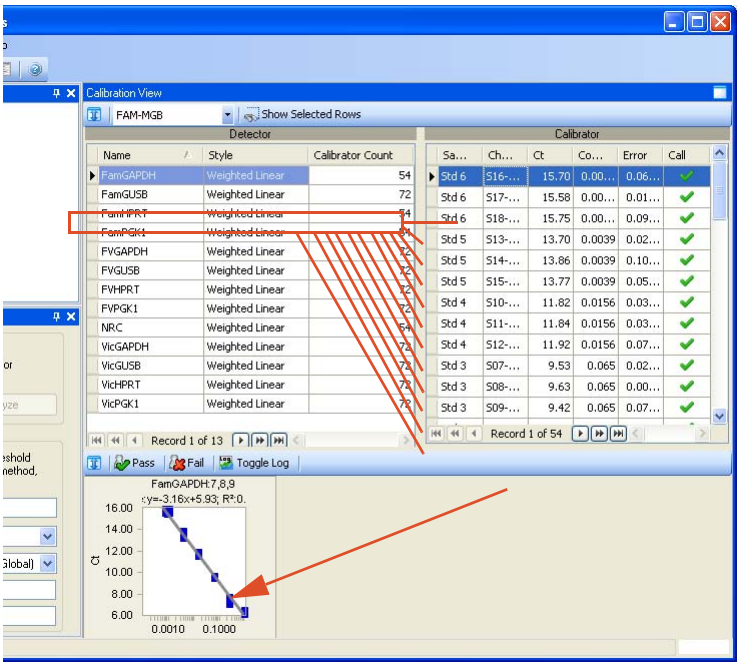
2 On the CCVM page, click on a detector in the Detector Table pane.

The Detector Table displays the attributes of a detector in three columns: Name of detector, Style of fitting method and Count of Calibrators for this row.

If detectors are named the same name, they are listed on one row in the table and the total of all calibrators are listed in the third column.

3 Adjacent to the Detector Table is a list of the calibrators applied to that detector. In the graph area below, valid detectors are plotted.

The Calibrator Table displays six attributes of a calibrator: Name, Chamber ID, C_t value, Concentration, Error, and Call.



Select a detector and its corresponding calibrators are listed on the right pane. The selected experiments are plotted on the graph area below.

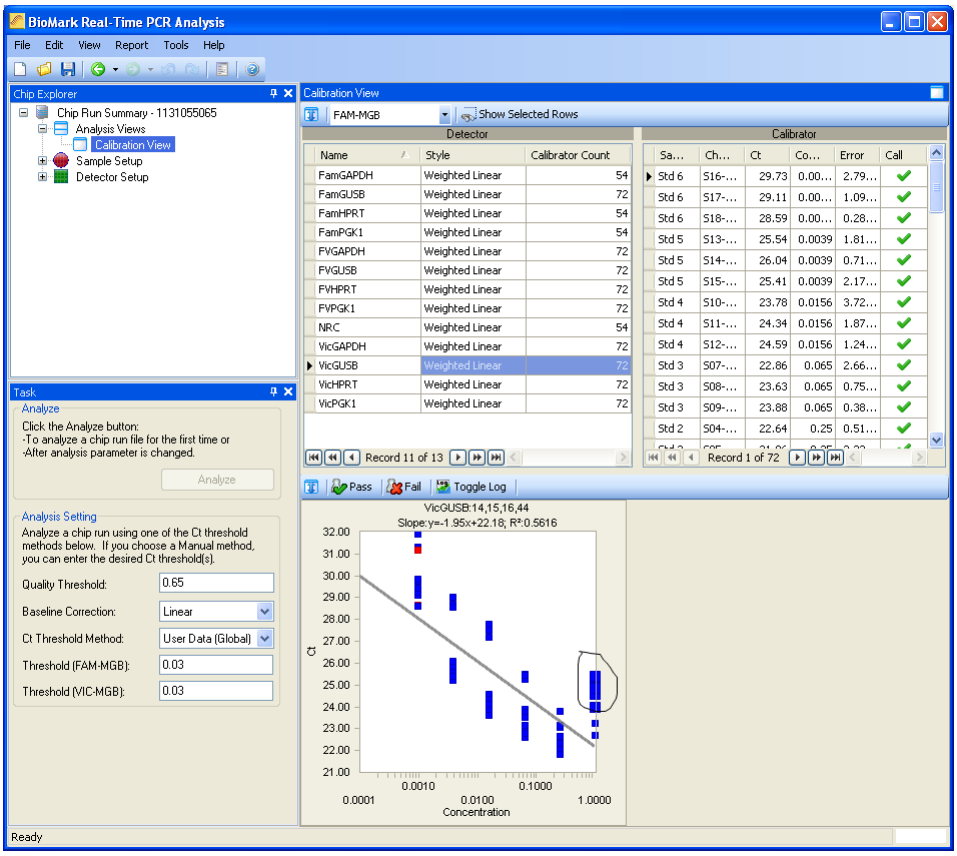
4 Select a row in the Calibrator Table and the corresponding data point in the calibration curve becomes larger. Conversely, you can lasso or click on a data point in the chart and the corresponding row in the Calibration Table is highlighted.

NOTE: You can lasso a point by pressing the left mouse button and dragging the mouse around the data point(s) to create a circle.

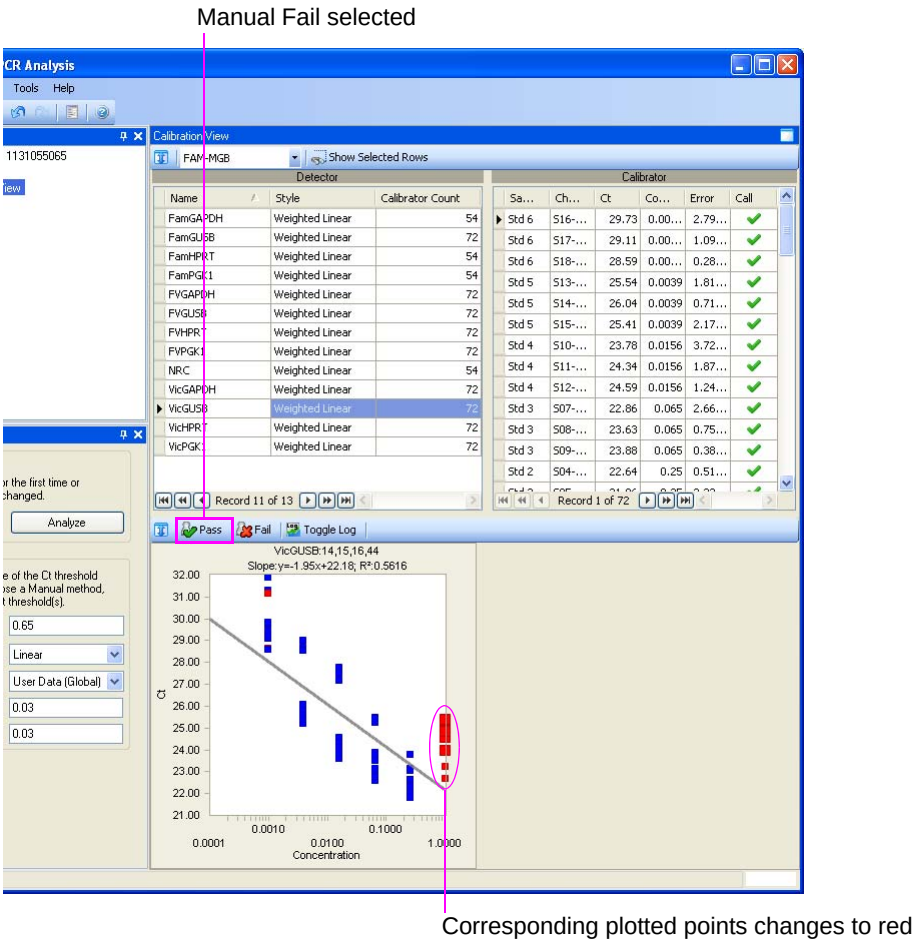
Only calibrators with valid C_t values are plotted in the calibration curve. Invalid C_t values are listed as 999. Calibrators that are auto or manual passed are plotted as blue dots. They are considered valid calibrators. Calibrators that are manually failed or passed are plotted as red dots and are considered invalid calibrators. CCVM only uses blue data points to create calibration curves. If there are no valid calibrators, no calibration curve is drawn.

5 You can modify the calls by manually changing the calibrators' calls to Pass or Fail via the secondary view tool bar.

a Select a calibrator you wish to change (you can select the row in the Calibrator Table or lasso a data point on the chart).



b Click either Pass or Fail on the secondary tool bar. Fail turns the points red. Pass turns the points blue. The corresponding calibrator in the Calibration Table changes its call accordingly and the Call column is updated.

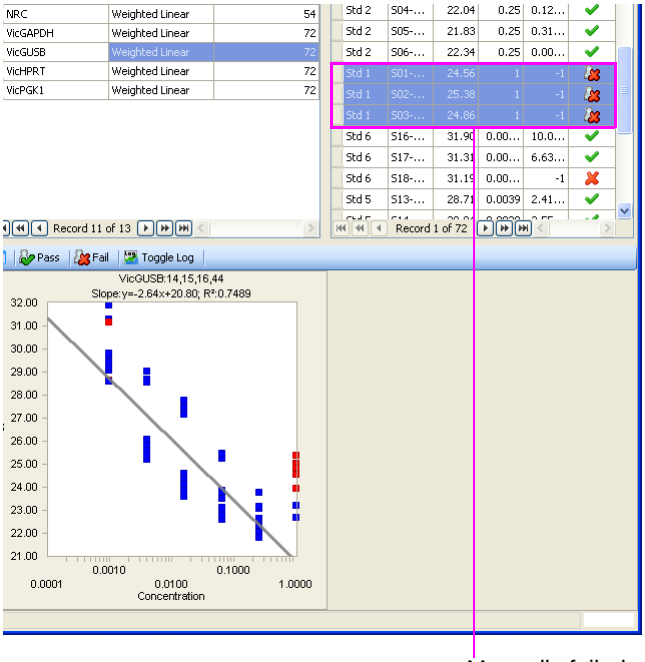


NOTE: You can use the Call Redo or Undo buttons to revert back to the original call state.

c Go to the **Analysis Views** page.

d Click the **Analyze** button to re-analyze the chip with these new parameters.

e The resulting calibration curve is slightly modified.

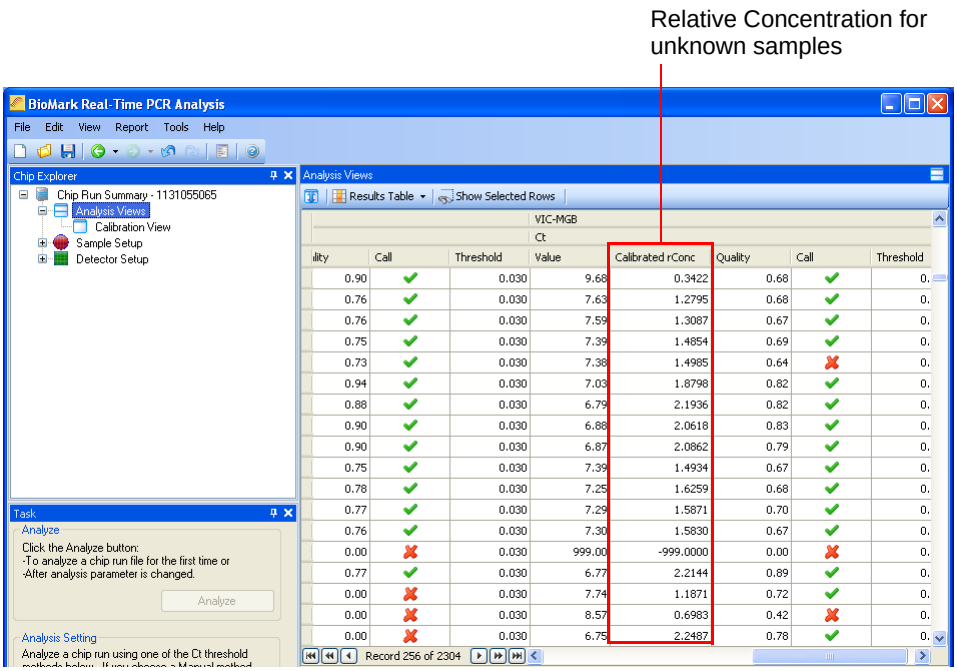


The analysis software can now use the calibration curve data to predict the approximate concentration of unknown sample types.

6 Go to the **Analysis Views** page.

7 Select the **Results Table**.

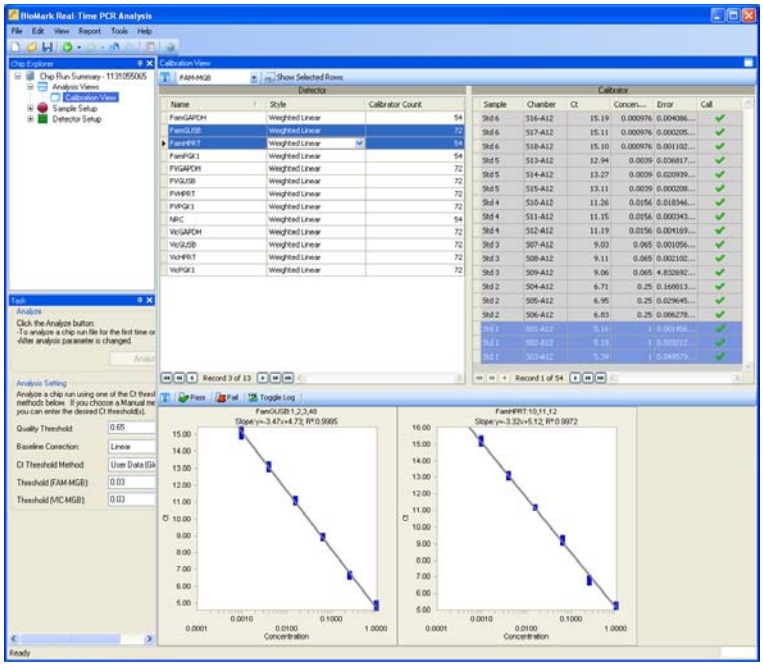
8 The approximate values are listed in the “Calibrated rConc” (Calibrated relative concentration) column.



Viewing Multiple Calibration Curves

You can also view multiple calibration curves at once on the CCVM page. To select multiple rows of assay in the Detector Table, Ctrl + left mouse click the rows of interest. Individual curves are rendered in the Graph Area.

Note, however, that the Calibrator Table behaves differently when multiple rows are selected in the Detector Table. The Calibrator Table is masked gray and the user cannot perform any actions on it. This is because the Calibrator Table was designed to only show calibrators for one detector at a time.



48.48 Dynamic Array™ IFC Real-Time PCR Workflow

A

In this appendix:

Priming the 48.48 Dynamic Array™ IFC

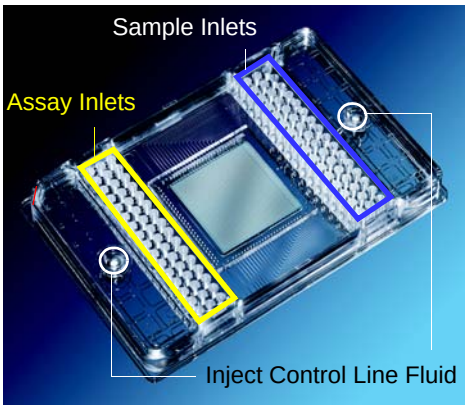
CAUTION! Use the 48.48 chip within 24 hours of opening the package.

- Due to different accumulator volumes, only use 48.48 syringes with 300 µL of control line fluid.
- Control line fluid on the chip or in the inlets makes the chip unusable.
- Load the chip within 60 minutes of priming.

1 Inject control line fluid into each accumulator on the chip.

2 Place the chip into the IFC (Integrated Fluidic Circuit) controller.

3 Using the IFC controller software, run the ***Chip Prime (113x)*** script to prime the control line fluid into the chip.



Preparing 10x Assays

In a DNA-free hood, prepare 5µL aliquots of 10X assays using the volumes in the table below (scale up appropriately for multiple runs).

Component	Volume (µL)
20x TaqMan® Gene Expression Assay (Applied Biosystems)	2.5
2x Assay Loading Reagent (Fluidigm, PN 85000736)	2.5
Total Volume	**5.0**
Final Concentration (at 10x)	**Primers: 9 µM; Probe: 2 µM**

Preparing Sample Pre-Mix and Samples

1 Combine the components in the table below to make the Sample Pre-Mix (first two rows) and the final Sample Mixture. (Scale up appropriately for multiple runs.)

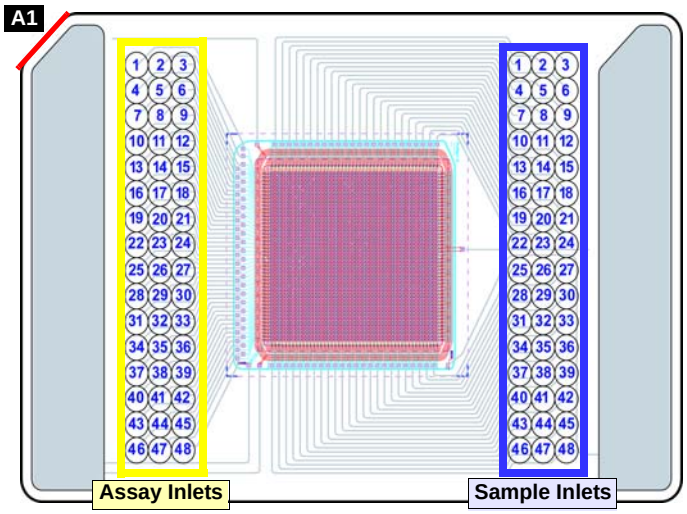
Sample Pre-Mix

Component	Volume per Inlet (µL)
TaqMan® Universal PCR Master Mix (2x) (Applied Biosystems, PN 4304437)	2.5
20x GE Sample Loading Reagent (Fluidigm, PN 85000735) ●	0.25
cDNA	2.25
Total Volume	**5.0**

2 In a DNA-free hood, combine the TaqMan Universal PCR Master Mix with the 20X GE Sample Loading Reagent in a 1.5 mL sterile tube—enough volume to fill an entire chip. 2.75µL of this Sample Pre-Mix can then be aliquoted for each sample.

3 Remove these aliquots from the DNA-free hood and add 2.25µL of cDNA to each, making the total volume of 5µL in each aliquot.

Chip Pipetting Map



Loading the Chip

IMPORTANT: Make sure you thoroughly mix all assay solutions and all samples before pipetting into the chip inlets.

1 When the *Chip Prime (113x)* script has finished, remove the primed chip from the IFC controller and pipette 5 µL of each assay and each sample into the respective inlets on the chip.

IMPORTANT: For unused sample inlets, use 2.75µL of Sample Pre-Mix and 2.25µL of DNA-free water per inlet. For unused assay inlets, use 2.5µL assay loading reagent and 2.5 µL of water.

IMPORTANT: Run NTC in sample inlet #22.

CAUTION! While pipetting, *do not* go past the first stop on the pipette— doing so may introduce air bubbles into inlets.

2 Return the chip to the IFC controller.

3 Using the IFC controller software, run the ***Load-Mix (113x)*** script to load the samples and assays into the chip.

4 When the *Load-Mix (113x)* script has finished, remove loaded chip from the IFC controller.

5 Peel the blue protective film from the underside of the loaded chip.

6 Remove any dust particles or debris from the chip surface.

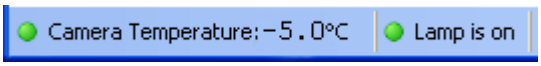
You are now ready to run the chip.

CAUTION! Start the chip run on the BioMark instrument within 4 hours of loading the samples.

Using the Data Collection Software

IMPORTANT: Be sure to select *all* probe types present in your experiment. Data are not collected on unspecified probes.

1. Double-click the Data Collection Software icon on the desktop to launch the software.
2. Click **Start a New Run**.
3. Check the status bar to verify that the lamp and camera are ready. Make sure both are green before proceeding.
4. Place the loaded chip into the BioMark reader.



5. Click **Load**.
6. Verify chip barcode and chip type.
   a. Choose project settings (if applicable).
   b. Click **Next**.
7. Chip Run file:
   a. Select **New** or **Predefined**.
   b. Browse to a file location for data storage.
   c. Click **Next**.
8. Application, Reference, Probes:
   a. Select Application—**Gene Expression**.
   b. Select Passive Reference (ROX).
   c. Select Assay—**Single probe**, **Two probes** or **More than two probes**.
   d. Select probe types.
   e. Click **Next**.
9. Click **Browse** to find thermal protocol file—**Default-10min-HotStart.pcl**.

CAUTION! Make sure that you use a 48.48 specific protocol.

10. Confirm Auto Exposure is selected.
11. Click **Next**.
12. Verify the chip run information.
13. Click **Start Run**.

Using the Real-Time PCR Analysis Software

IMPORTANT: Click **Analyze** after making changes to any parameter.

1. Double-click the Real-Time PCR Analysis software icon on the desktop to launch the software.
2. Click **Open Chip Run**.
3. Double-click a **ChipRun.bml** file to open it in the software.
4. Select **Analysis Views** in the chip explorer pane.
5. Click **Analyze**.
6. View data in three different modes:

- Results Table
- Image View
- HeatMap.
- There are additional views in the Graph View for each mode.

7. Export data from the Results Table and/or the Heat Map using .csv format.
8. Choose a view from the drop-down menu:

- Results Table
- Image View
- Heat Map View

96.96 Dynamic Array™ IFCs Real-Time PCR Workflow

B

In this appendix:

Priming the 96.96 Dynamic Array™ IFC

CAUTION! Use the **96.96** chip within 24 hours of opening the package.

- Due to different accumulator volumes, only use 96.96 syringes with 150µL of control line fluid.
- Control line fluid on the chip or in the inlets makes the chip unusable.
- Load the chip within **60** minutes of priming.

1. Inject control line fluid into each accumulator on the chip.
2. Place the chip into the IFC (integrated fluidic circuit) controller then, using the IFC controller software, run the ***Chip Prime (136x)*** script to prime the control line fluid into the chip.

Preparing 10x Assays

In a DNA-free hood, prepare 5 µL aliquots of 10x assays using the volumes in the table below (scale up appropriately for multiple runs).

Component	**Volume (µL)**
20x TaqMan® Gene Expression Assay (Applied Biosystems)	2.5
2x Assay Loading Reagent (Fluidigm, PN 85000736)	2.5
Total Volume	**5.0**
Final Concentration (at 10x)	Primers: 9 µM; Probe: 2 µM

Preparing Sample Pre-Mix and Samples

1 Combine the components in the table below to make the Sample Pre-Mix (first two rows) and the final Sample Mixture (scale up appropriately for multiple runs).

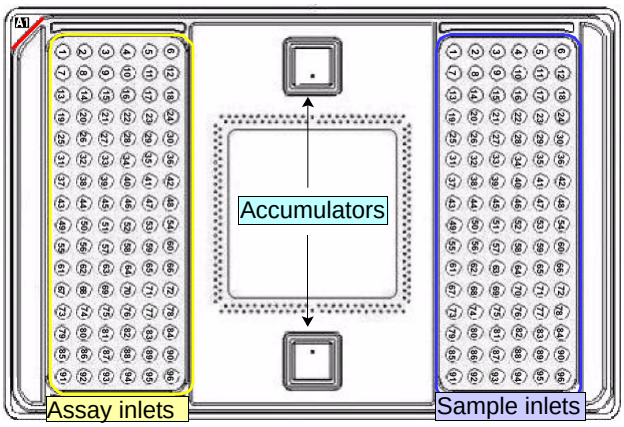
Component	Volume per Inlet (µL)
TaqMan® Universal PCR Master Mix (2x)(Applied Biosystems PN 4304437)	2.5
20x GE Sample Loading Reagent (Fluidigm, PN 85000735)	0.25
cDNA	2.25
Total Volume	**5.0**

Sample Pre-Mix

2 In a DNA-free hood, combine the TaqMan Universal PCR Master Mix with the 20X GE Sample Loading Reagent in a 1.5 mL sterile tube—enough volume to fill an entire chip. 2.75 µL of this Pre-Sample Mix can be aliquoted for each sample.

3 Remove these aliquots from the DNA-free hood and add 2.25 µL of cDNA to each, making a total volume of 5 µL in each aliquot.

Chip Pipetting Map



Loading the Chip

IMPORTANT: Make sure you thoroughly mix all assay solutions and all samples *before* pipetting into the chip inlets.

1 When the *Chip Prime* (*136x)* script has finished, remove the primed chip from the IFC controller and pipette 5 µL of each assay and each sample into their respective inlets on the chip.

IMPORTANT: For unused sample inlets, use 2.75 µL of sample mix and 2.25 µL of water per inlet. For unused assay inlets, use 2.5 µL assay loading reagent and 2.5 µL of water.

CAUTION! While pipetting, *do not* go past the first stop on the pipette. Doing so may introduce air bubbles into inlets.

2 Return the chip to the IFC controller.

3 Using the IFC controller software, run the ***Load-Mix (136x)*** script to load the samples and assays into the chip.

4 When the *Load-Mix* (*136x)* script has finished, remove loaded chip from the IFC controller.

5 Peel the blue protective film from the underside of the loaded chip.

6 Remove any dust particles or debris from the chip surface.

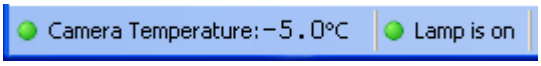
You are now ready to run the chip.

CAUTION! Start the chip run on the instrument immediately after loading the samples.

Using the Data Collection Software

IMPORTANT: sure to select *all* probe types present in your experiment. Data are not collected on unspecified probes.

1. Double-click the Data Collection Software icon on the desktop to launch the software.
2. Click **Start a New Run**.
3. Check the status bar to verify that the lamp and the camera are ready. Make sure both are green before proceeding.



4. Place the loaded chip into the BioMark reader.
5. Click **Load**.
6. Verify chip barcode and chip type.
   a. Choose project settings (if applicable).
   b. Click **Next**.
7. Chip Run file:
   a. Select **New** or **Predefined**.
   b. Browse to a file location for data storage.
   c. Click **Next**.
8. Application, Reference, Probes:
   a. Select Application—**Gene Expression**.
   b. Select Passive Reference (ROX).
   c. Select Assay—**Single probe**, **Two probes** or **More than two probes**.
   d. Select probe types.
   e. Click **Next**.
9. Click **Browse** to find thermal protocol file—**M96 default protocol.pcl**.

CAUTION! Make sure that you use a **96.96** specific protocol.

10. Confirm Auto Exposure is selected.
11. Click **Next**.
12. Verify the chip run information.
13. Click **Start Run**.

Using the Real-Time PCR Analysis Software

IMPORTANT: Click **Analyze** after making changes to any parameter.

1. Double-click the Real-Time PCR Analysis software icon on the desktop to launch the software.
2. Click **Open Chip Run**.
3. Double-click a **ChipRun.bml** file to open it in the software.
4. Select **Analysis Views** in the chip explorer pane.
5. Click **Analyze**.
6. View data in three different modes:

- Results Table
- Image View
- HeatMap.
- There are additional views in the Graph View for each mode.

7. Export data from the Results Table and/or the Heat Map using .csv format.
8. Choose a view from the drop-down menu:

- Results Table
- Image View
- Heat Map View

48.48 PCR Installation Test

C

In this appendix:

Overview

This test distinguishes a two-fold change in the cDNA copy number with a 99.7% confidence level within the same chip, using real-time quantitative PCR. Real-time quantitative PCR is run and data are analyzed using the system's standard algorithms.

For each chamber, the analysis software generates either a C_t or a No-Call. The pass criteria are:

- A standard deviation of $C_t \leq 0.25$ for each group (1X and 2X).
- A maximum of 0.5% No-Calls per chip.
- Difference of mean C_t between groups of 1.00 +/- 0.10.

IMPORTANT: Start the Data Collect Software 20 minutes before starting the run to allow the lamp time to warm to optimum temperature.

IMPORTANT: If the lamp has automatically turned off due to inactivity (after approximately 2 hours), reset it. For instructions on resetting the lamp see the *BioMark Data Collection User Guide* (PN 68000080).

Sample and Reagent Processing

- To prevent carry-over of amplified DNA sequences, set up PCR reactions in a separate containment area (such as a laminar flow hood) from that used for post-PCR manipulations.
- Prepare reagents in the "DNA-free" laminar flow hood. This area is to be kept free of **any biological material,** including DNA/RNA extracts and PCR products.
- Procedures carried out in this area include preparation and aliquoting of reagent stocks and preparation of reaction mixes prior to the addition of the sample nucleic acid.
- Extracted DNA is to be added to the PCR reaction mixes in the "Sample" laminar flow hood. The PCR reaction mixes should first be prepared in the "DNA-free" laminar flow hood.
- If possible, include a positive control which amplifies weakly but consistently. The use of a strongly positive control sample could result in an excess of amplified product which serve as a source of contamination.
- Use well characterized negative samples, such as lambda DNA.
- Include no template negative controls containing all the necessary reagent components but excluding test DNA.

- Two PCR workstations like the one shown. Visit http://www.vwrsp.com.

- Filtered pipette tips.
- Calibrated pipettes.

Reagents Required

Reagents stored at -20°C

- cDNA (Fluidigm PN 81000190)
- 20X GAPDH reagent (Applied Biosystems PN4333764F)

Reagents stored at 4°C

- Taqman® Universal PCR Master Mix (Applied Biosystems PN 4304437)
- 20X GE Sample Loading Reagent (Fluidigm PN 85000735)
- 2X Assay Loading Reagent (Fluidigm PN 85000736)

Reagents stored at room temperature

- Deionized DNA-free, DNase-free, RNase-free water (Fluidigm PN 81000204)
- TE: 10 mM TrisHCl, 0.1 mM EDTA, pH 8.0 (Technova, catalog # T0221)

Preparing the Chip

Place the chip in the IFC Controller.

1. Launch the IFC Controller Software.
2. From the Script menu, select **Chip Prime (113x)**.
3. Click **Start**.
4. Once the chip has finished priming, remove it from the IFC Controller.

Preparing Assays

Add 2.5 µl of 2X Assay Loading Reagent to 2.5 µl of 20X GAPDH reagent. Total = 5.0 µl per inlet.

Pipette 5.0 µl of the reagent aliquot into each detector inlet of the chip.

Preparing the Sample Mix

IMPORTANT: Be sure to use the DNA-free hood when preparing the reagents.

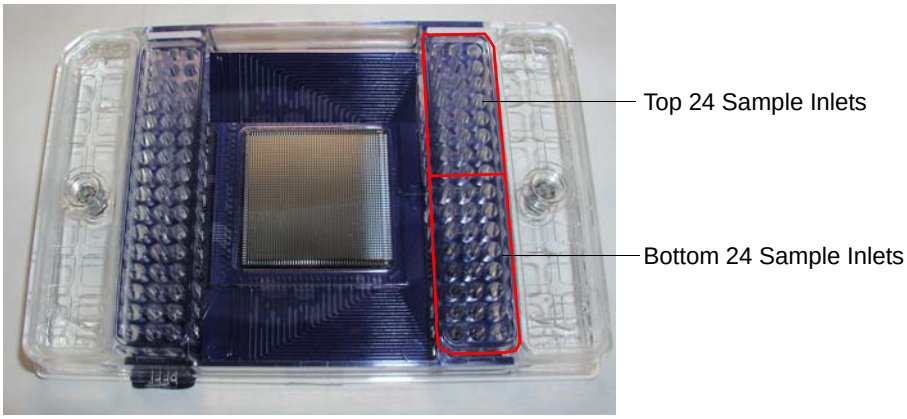
Prepare the Sample Mix by combining the following in a 1.5 mL sterile tube (for a single chip run):

1. 244.4 µl Taqman® Universal PCR Master Mix (Applied Biosystems PN 4304437)
2. 24.4 µl 20X GE Sample Loading Reagent (Fluidigm PN 85000735)
3. 61.2 µl TE: 10 mM TrisHCl, 0.1 mM EDTA, pH 8.0 (Technova, catalog # T0221)
   - **Final volume**: 330 µl
4. Remove the tube from the hood and vortex for at least 20 seconds.
5. Split the Sample Mix into two 500 µl tubes, each with 150 µl of the Sample Mix.
6. Label one tube 1X and the other 2X.
7. Add 25 µl of TE buffer to the 1X tube.
8. Add 25 µl of the aliquotted cDNA (Fluidigm PN 84000190) to the 1X tube.
9. Add 50 µl of the aliquotted cDNA (Fluidigm PN 84000190) to the 2X tube.

Preparing Samples

IMPORTANT: Be sure to use the Sample hood when preparing samples.

1. Pipette 5.0 µl of 1X Sample Mix into each of the top 24 sample inlets on the chip.
2. Pipette 5.0 µl of 2X Sample Mix into each of the *bottom* sample inlets on the chip.

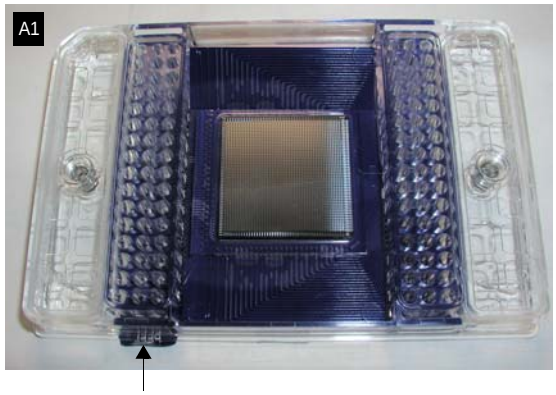


NOTE: While pipetting, do not go past the first stop on the pipette—doing so may introduce air bubbles into the inlets.

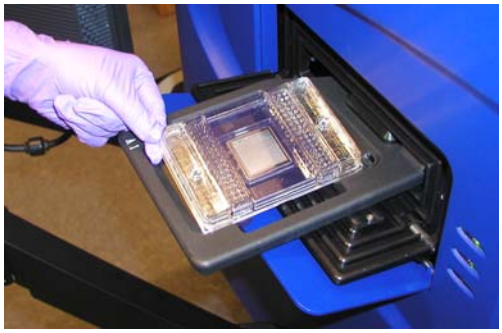
Loading the Chip

1. Place the chip in the IFC Controller.

2. Launch the IFC Controller Software.
3. Select the **Load-Mix (113x)** script.
4. Click **Start**.
5. Once the chip has finished loading, remove it from the IFC Controller, and inspect it for any obvious loading defects.
6. Remove the blue plastic protector from beneath the chip.
7. Place the chip into the BioMark reader.

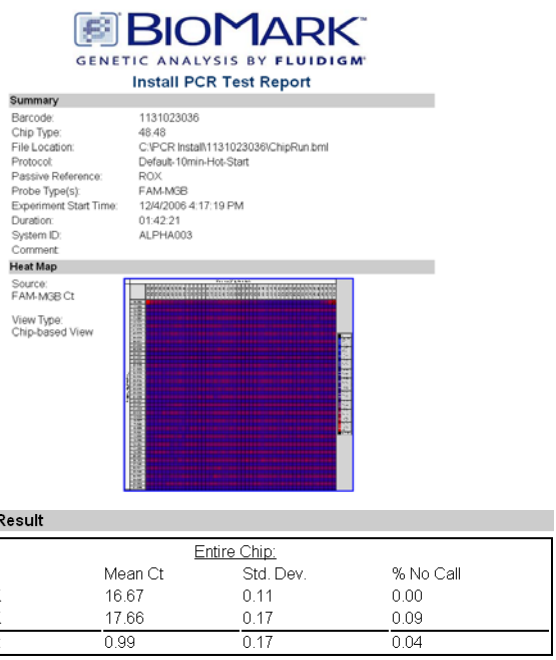


Remove the blue plastic film from beneath the chip



Begin the PCR Installation Test

1. Launch the Data Collection software.
2. Click **Start a New Run**.
3. Check the status bar to verify that the lamp and camera are ready.
4. Place the loaded chip into the BioMark reader.
5. Click **Load**.
6. Verify chip barcode and chip type.
   a. Choose project settings (if applicable).
   b. Click **Next**.
7. Chip Run file:
   a. Select **New** or **Predefined**.
   b. Browse to a file location for data storage.
   c. Click **Next**.
8. Application, Reference, Probes:
   a. Select Application Type—**Gene Expression**.
   b. Select Passive Reference (**ROX**).
   c. Select Assay—**Single Probe**, **Two Probes** or **More than two probes**.
      - Select probe types**.**
   d. Click **Next**.
9. Click **Browse** to find thermal protocol file—**Default-10min-HotStart.pcl**.
10. Confirm **Auto Exposure** is selected.
11. Click **Next**.
12. Verify the chip run information.
    - Click **Start Run**.
13. After the run is completed, process the data:
    a. Launch BioMark Real-Time PCR Analysis software and process the images.
    b. Select **PCR Test Report**. The report indicates whether the chip has passed the install test (see the example below).



BIOMARK
GENETIC ANALYSIS BY FLUIDIGM

Install PCR Test Report

Summary

Barcode:	1131023036
Chip Type:	48.48
File Location:	C:\PCR Install\1131023036\ChipRun.bml
Protocol:	Default-10min-Hot-Start
Passive Reference:	ROX
Probe Type(s):	FAM-MGB
Experiment Start Time:	12/4/2006 4:17:19 PM
Duration:	01:42:21
System ID:	ALPHA003
Comment:	

Heat Map

Source: FAM-MGB Ct

View Type: Chip-based View

PCR Test Result

	Entire Chip:		
	Mean Ct	Std. Dev.	% No Call
Sample 2X	16.67	0.11	0.00
Sample 1X	17.66	0.17	0.09
Test Result	0.99	0.17	0.04

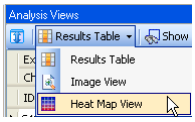
Passing the Test with Outliers

Up to 0.5% No-Calls are permitted in a passing PCR Installation Test. This translates to 11 out of 2304 chambers on a Fluidigm 48.48 Dynamic Array™ IFC (Integrated Fluidic Circuits).

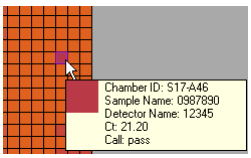
If high- or low-C_t outliers are present but have not been rejected by the curve quality detection algorithms, it is permissible to manually convert high- or low-C_t outliers to No-Calls. Follow the procedure below to change the outliers to No-Calls.

Changing Outliers to No-Calls

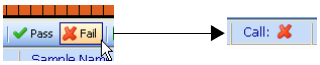
1. In the BioMark Real-Time PCR Analysis Software, open the .bml chip run file that you want to modify.
2. Click **Analysis Views**.
3. Click **Heat Map** in the Results Table drop-down menu.



4. Click the outlier to be changed.

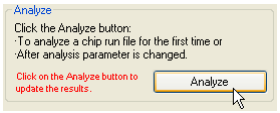


5 Click **Fail** to change the cell to No-Call.

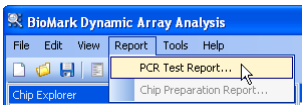


The change is reflected in the Heat Map as an X in the cell.

6 Click **Analyze**.



7 Click **Report > PCR Test Report** to generate a report that reflects your changes.



Congratulations, the install test is complete.

World Headquarters
7000 Shoreline Court, Suite 100
South San Francisco, CA 94080
USA

Fluidigm Europe, BV
Parnassustoren
Locatellikade 1, 1076 AZ
Amsterdam
Netherlands
Tel: +31 (0) 20 578 88 53
Fax: +31 (0) 20 203 1111

Fluidigm Japan KK
Level 5, Ginza TK Building
1-1-7 Shintomi
Chuo-ku, Tokyo 104-0041 Japan
Office: +81335552351
Fax: +8133552353

Fluidigm Singapore PTE LTD
Block 1026 Tai Seng Avenue
#07-3532
Tel: (65) 6858 7316
Fax: (65) 6282 5531
fluidigm.singapre@fluidigm.com

Technical Support

send email to:
TechSupport@fluidigm.com

Phone in United States:
1.866.FLUIDLINE (1.866.358.4354)

Outside the United States:
650.266.6100

On the Internet:
www.fluidigm.com/support

Visit our website at
www.fluidigm.com